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A TECHNIQUE FOR ESTIMATING THE FECUNDITY OF NATURAL POPULATIONS OF THE SPRUCE BUDWORM[1]

C. A. MILLER[2]

Abstract

In a severe infestation late-instar spruce budworm larvae that normally feed on current foliage may be forced to complete development on old foliage. This results in a reduction in fecundity. The precise causes of this reduction are not known, but factors that may be involved are the amount and the age of the old foliage consumed. The relationship of pupal case size and fecundity is used as a basis for estimating the expected fecundity in a natural population under these conditions of partial larval starvation. Three regression equations applicable to conditions on the Green River area, New Brunswick, are presented. An index of the actual increase of a population is obtained from an E/F ratio. Ratios may be compared in order to indicate some aspects of oviposition. Actual and expected egg populations can also be compared, subject to certain limitations, to indicate some aspects of adult dispersal. The mean number of eggs per mass in relation to degree of infestation and a simplified method of counting eggs per mass are also discussed.

Introduction

This is the fifth in a series of papers (4, 6, 3, 5) dealing with techniques used in ecological studies of the spruce budworm, *Choristoneura fumiferana* (Clem.), on the Green River Watershed in northwestern New Brunswick. It elaborates on the methods used in developing that part of a life table for the spruce budworm (6) dealing with the pupal and adult stages.

At low population levels late-instar budworm larvae normally feed on the current year's growth of the host tree. During a severe infestation, however, these larvae may be forced to feed on old foliage owing to the destruction of the current year's growth before the completion of larval development. A similar condition can also occur at moderate population levels on balsam fir, *Abies balsamea* (L.) Mill., if the normal foliage production of the tree is reduced either by late-spring frosts or flower production. Studies in the Green River area have shown that pupal size is related to fecundity and that both factors decrease in a population forced in successive generations to complete its larval development on old foliage. The ultimate values of these factors may be influenced by a number of conditions including: (1) the degree of forced feeding on old foliage and (2) the age of the old foliage consumed. Defoliation

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patterns indicate that, during the first year of a severe infestation, forced feeding on old foliage is mainly confined to one- and two-year-old shoots. This causes a reduction in fecundity that is apparently in direct ratio to the reduction in pupal size and is therefore not significantly different from the expected trend. If the degree of infestation remains high and the current foliage is completely consumed each year, the population will be forced to feed on progressively older foliage. This condition is coincident with a reduction in fecundity that is significantly different from the expected trend. The objective of the technique to be described is to estimate the fecundity in a population where the degree and age of the infestation are known.

Blais, in both laboratory (2) and field studies (1), found a decrease in the fecundity of spruce budworm adults issuing from a population that was forced to feed on old foliage. He also indicates that the fecundity of the same population will increase again if larval development can be completed on current year's growth. In a monograph on the nun moth in Germany (10), Mors cites data to show changes in the fecundity of this insect in four successive generations. He uses the regression of fecundity on pupal size to indicate that these changes cannot be attributed to pupal size alone. Instead, he attributes the differences in fecundity to 'intrinsic' influences brought about by the gradual weakening in the 'constitution' of the population. It is therefore apparent that changes in the reproductive rate of a population do occur and such changes may have a decided influence on population trend. Further, these changes are not necessarily restricted to severe infestations, but observed differences in mean pupal size indicate that they may also occur in non-starved populations. Thus, the policy of applying an average fecundity to a non-starved population year after year, as inferred by Morris and Miller (6), may not be valid, and this constitutes the primary reason for developing a technique to obtain successive measurements of field fecundity.

The terms 'fecundity', 'actual increase', 'actual eggs', and 'pupal size' as used in this paper are defined as follows. 'Fecundity' refers to the number of eggs laid by a female budworm. The spruce budworm will mate and oviposit satisfactorily under cage conditions, and fecundity can be measured in the laboratory. 'Actual increase' refers to the increase in density between the pupal and egg stage in a field population. The actual increase is expressed as an E/F ratio, the ratio, per unit area of foliage, of the number of eggs to the number of female pupal cases showing successful adult emergence (9). This ratio permits a comparison of actual increases between plots, or between years on the same plot, when differences in pupal density occur. 'Actual eggs' refers to number of eggs laid by a field population per unit area of foliage. The use of actual eggs rather than actual increase is the more direct procedure when comparing actual and expected egg populations on one plot when the pupal density is constant. The term 'pupal size' refers to a linear measurement of the depth of the first completely visible segment of the pupa or pupal case posterior to the wing pads. A female budworm pupa has four visible segments posterior to the wing pads, of which the 'first' may be partially covered by the tip of the wing pad while the 'second' is completely visible.

The depth of this segment along the dorsoventral plane is taken as the index of pupal size. The measurement is made along the dorsoventral plane since a living pupa normally curls ventrally while resting on a flat surface. There is little difference between this measurement and a similar measurement of the empty pupal case.

The following plots are referred to in brief examples of the application of the technique.

Plot K1.—A mature, predominantly balsam fir stand which was the apparent epicenter of an infestation first classified as severe in 1950. This infestation has caused 100% defoliation of current shoots and partial defoliation of old shoots each year since 1950. By the fall of 1954, 6% of the balsam fir (d.b.h., 4 in. and over) were dead, with an increasing rate of mortality expected in the near future.

Plot K2.—A small, dense stand of 35-year-old balsam fir, adjacent to Plot K1 and within the same infestation. Balsam fir mortality 0.5% (d.b.h., 4 in. and over).

Plot G2.—A mature, predominantly balsam fir stand outside the perimeter of the present severe infestation on the Green River Watershed.

Plot G9.—A mature, predominantly balsam fir stand severely infested in 1952 and sprayed with DDT in 1953.

Pupal Size Versus Pupal Weight as a Basis for Estimating Fecundity

The fecundity of a spruce budworm can be estimated either on the basis of pupal weight or pupal size since both factors are related (Fig. 1) and are also related to fecundity. In experiments designed to estimate the fecundity of natural populations, it has been found that the use of pupal size has definite advantages in the study of natural populations.

Living budworm pupae are present in the field from late June to late July in the Green River area. The pupal stage is approximately 10 to 12 days in

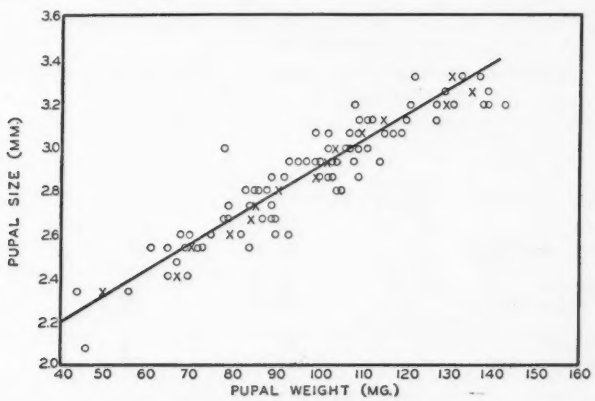

FIG. 1. Regression of pupal size on pupal weight for the spruce budworm obtained from 90 paired measurements, with mean values also indicated (x).

length and there is a definite range in time over which pupation occurs in any one year. For experimental purposes, pupae may be grouped into two classes to distinguish between early and late seasonal development. Fecundity, pupal weight, and frass weight associated with seasonal differences in the pupation of female pupae were investigated in the years 1948–1950 on plots where current defoliation was less than 100%. Low population levels made it necessary to collect late sixth-instar larvae in addition to pupae for these experiments.

Table I shows that individuals with an early seasonal development have a significantly greater fecundity and pupal weight than those with a late seasonal development. Preliminary experiments on frass yield[3] (Table I) indicate that female larvae pupating in early July yield a significantly greater weight of frass than those pupating later.

These data raise certain objections to the use of pupal weight in estimating fecundity for natural populations. Since weight is related to seasonal development, the mean weight for any one population would have to be obtained either at a precise period in pupal development or from a series of weighted means. The latter method is cumbersome. Presumably, under the first method, the mean weight would be obtained at the peak of the pupal stage but this is not clearly defined because of a high degree of overlapping in the late-larval, pupal, and adult stages. It has also been observed, but not tested statistically, that the weight of any one pupa may vary under certain environmental conditions during the period of pupal development. The mean weight of a group of female pupae collected in the field was found to be greater than the mean weight of the same pupae after four days exposure to warm, relatively dry air, suggesting that climatic conditions as well as seasonal development may affect pupal weight. Another disadvantage in the use of pupal weight is that dead or parasitized individuals may be included in group measurements of apparently healthy specimens.

TABLE I

SEASONAL DIFFERENCES IN FECUNDITY, PUPAL WEIGHT, AND FRASS WEIGHT, BASED ON DATE OF PUPATION

	Early	Late	Mean difference
No. of experiments	28	26	
Mean fecundity per female	181.3	130.5	50.8*
No. of experiments	58	49	
Mean pupal weight (mg.)	99.9	78.3	21.6*
No. of experiments	10	4	
Mean frass weight (mg.)	240.8	188.5	52.3*

*Significant within the 1% level.

[3]S. A. H. Davis, Green River Annual Report, 1949.

The principal advantage of using pupal size is that a population mean can be obtained from measurements of pupal cases after adult emergence. It is then known that no 'abnormal' specimens are included in the sample nor is the sample biased by seasonal development. Another advantage is that pupal cases can be collected in conjunction with pupal population counts, and thus comprise a representative sample for all crown levels (5).

The Regression Equations

The regression of fecundity on pupal size forms the basis of the technique for estimating the fecundity of natural populations of the spruce budworm. The primary assumption is that, having established this regression from oviposition experiments for a population with a known infestation history, the fecundity of another population in the same area and with a similar infestation history can be estimated from measurements of pupal size. Thus, three regression equations applicable to conditions encountered during the first five years of severe infestation were obtained from oviposition experiments. Living female pupae were collected on permanent sampling plots of known infestation history and placed singly in emergence jars. After adult emergence, the pupal case was measured and the adult set up in an oviposition cage with a single male. The cage contained a balsam twig approximately 6 in. long, set in a metal container filled with water and enclosed by a lantern globe. When the female was no longer active the egg masses were removed from the foliage and counted. Experiments were ignored in which mating did not occur or only sterile masses were found, as sterile masses are a rarity in field observations. The pupal cases were measured to the nearest tenth of a millimeter with an ocular micrometer in a stereoscopic microscope.

TABLE II

REGRESSION ANALYSIS OF FECUNDITY ON PUPAL SIZE FOR THE SPRUCE BUDWORM

The three series of experiments are based on degree of infestation

	A Normal	*B* Severe infestation "4th" year	*C* Severe infestation "5th" year
No. experiments	86	71	70
Mean pupal size (mm.)	2.84	2.62	2.73
Mean fecundity	156.5	110.8	99.0
Regression coefficient	133.69	135.85	102.58
Correlation coefficient	.76	.81	.72
Adjusted regression coefficient	125.82	125.82	125.82
Standard error of estimate	39.88	26.46	28.52
Analysis to test deviations from linear regression	$F = 1233.7/1657.7$	$F = 661.1/769.6$	$F = 1059.5/765.7 = 1.38$ ($F_{.05} = 2.49$)

Adjusted regression equation: (*A*) $y = 125.82x - 200.83$
(*B*) $y = 125.82x - 218.85$
(*C*) $y = 125.82x - 244.49$

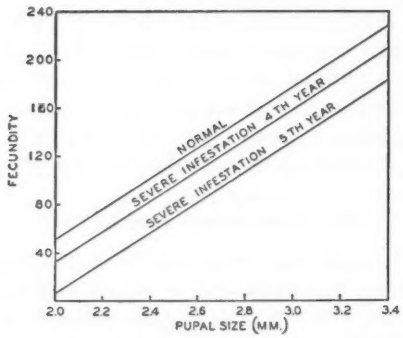

FIG. 2. Regressions of fecundity on pupal size for the spruce budworm in three degrees of infestation. Lines based on the average regression coefficient.

The following three series of experiments were conducted in this manner (Table II):

Series A.—Female pupae were collected from plots where the current defoliation was less than 100% and from a second group of plots where feeding on old foliage had occurred for a maximum of two years. This was necessary to obtain a wide range in pupal size. The regression line of fecundity on pupal size obtained from these combined data is termed the 'normal' regression line (Fig. 2).

Series B.—Female pupae were collected in an area (Plot K1) that had been severely infested for four consecutive years. Population pressure each year forced the larvae to feed on old foliage. The regression line for these data is termed the '4th' year in Fig. 2.

Series C.—Female pupae were collected in the same area as for series *B* in the 5th year of severe infestation. A drop in current foliage production in addition to population pressure again caused a considerable degree of feeding on old foliage. The regression line is termed the '5th' year in Fig. 2.

An analysis of covariance of the three series of experiments shows significant differences in fecundity after adjustment for differences in pupal size. A further test shows the difference between 'normal' and '4th year' to be significant within the 5% level, and between 'normal' and '5th year' within the 1% level. The same analysis shows no significant differences among the three regression coefficients and it is therefore permissible, as shown in Table II, to use an average regression coefficient in the regression equations as the best available estimate of population regression. The deviation from linearity for each regression line was also tested by an analysis of variance (8). Table II shows that, in series *A* and *B*, the mean square corresponding to deviations from linear regression is slightly less than that for error and, in series *C*, no significant departure from linearity is indicated.

It is beyond the scope of this paper to discuss in detail the factors involved in the changes in fecundity. The tests show that factors other than pupal size are in operation. One obvious difference in the three series of experiments

is the age of the foliage which the larval population was forced to consume to complete development. Until further data are gathered on the spruce budworm the degree and age of an infestation will be treated as indicators of reduced fecundity and not precise causal agencies. It is interesting to note that fecundity increased for a number of individuals that were collected as II- and III-instar larvae from Plot K1 in 1954 and again in 1955, and reared in the insectary on an abundant supply of new foliage. The results of 38 oviposition experiments in 1954 could not be tested with the 'normal' regression line because of a significant difference between regression coefficients. Fecundity was higher than normal for large pupae and lower than normal for small pupae. Very similar results were obtained from 62 experiments in 1955, which permitted the grouping of the two years' data. The difference in regression coefficients indicates that the possible relationship between intrinsic factors and changes in fecundity must also be considered in a declining infestation. This will be the subject of more detailed work in the future.

Application of Regressions

Pupal cases are collected in conjunction with the pupal population sampling on each plot. The female cases are separated from the sample and measured in order to obtain mean pupal size. The expected fecundity is then estimated by substituting mean pupal size in the appropriate equation, depending on the history of the infestation.

Measurements of mean pupal size are obtained on all sampling plots, but, at very low population densities, when few pupae are collected, it is necessary to combine data for plots of similar infestation history. An analysis of case measurements shows that it requires approximately 60 measurements to establish a population mean with an acceptable error of 3.0% at the '$t_{.01}$' level (8).

Each year experimental checks are maintained on the 'normal' equation using pupae collected within the light infestation. Within the severe infestation area, where larval density and the degree of food shortage may change considerably from year to year, sufficient pupae are collected to carry out 70 to 100 oviposition experiments. These experiments either provide a check on existing equations, or data to establish a new equation to fit conditions existing at the time.

Actual Increases

The determination of the actual increase in a population requires, in particular, an intensive sampling of the egg stage. Spruce budworm eggs are laid in masses, and one useful method of counting field-collected material is by using the relationship of mass length to number of eggs rather than counting individual eggs in a mass. Table III shows the values read from a regression line fitted to the data for 417 typical egg masses, assuming that the typical mass on a balsam fir needle has two complete rows of eggs and a partial third row. It is doubtful whether the regression line is applicable to masses laid

TABLE III

RELATIONSHIP OF MASS LENGTH TO NUMBER OF EGGS FOR THE SPRUCE BUDWORM OVIPOSITING ON BALSAM FIR

Length (mm.)	Eggs	Length	Eggs	Length	Eggs
2.0	4	4.4	20	6.8	35
2.2	6	4.6	21	7.0	36
2.4	7	4.8	22	7.2	38
2.6	8	5.0	23	7.4	39
2.8	9	5.2	25	7.6	40
3.0	11	5.4	26	7.8	41
3.2	12	5.6	27	8.0	42
3.4	13	5.8	28	8.2	43
3.6	15	6.0	30	8.4	45
3.8	16	6.2	31	8.6	46
4.0	17	6.4	32	8.8	47
4.2	18	6.6	33	9.0	48

on white spruce needles, nor is it accurate for masses of less than five eggs. In the latter case, it is not difficult to count the individual eggs.

Egg counts in the Green River area indicate that mean eggs per mass is dependent on the degree of infestation; if a population is subject to larval starvation the resultant small adults tend to lay smaller masses. Table IV shows accumulated eggs per mass data from field and experimental material. The difference between mean eggs per mass for light and severe infestations is significant. In a general survey of egg populations these differences may be of little account, whereas in an intensive study of population trends, such differences should be taken into consideration.

The actual increase in a population is expressed as an E/F ratio. Varley (9) uses this relationship to measure fecundity when a population is not affected by migration. The ratio is calculated from counts of eggs and pupal cases expressed as population per 10 sq. ft. of branch surface and based on the same sample trees on each study plot (5). If pupal counts were correlated with egg counts, E/F ratios could be calculated for individual trees and a mean ratio estimated for the plot. However, no correlation exits between the two counts although both samples are obtained from the same trees (5). It is therefore preferable to combine the counts for all trees and obtain a mean pupal and a mean egg population for the plot. An E/F ratio is then calculated from these two means.

TABLE IV

SIGNIFICANT DIFFERENCES IN EGGS PER MASS AS RELATED TO DEGREE OF INFESTATION OR ADULT SIZE

	Source of material	No. of masses measured	Eggs per mass
Severe infestation	Field	996	15.7 ± 0.3
Light infestation	Field	7429	18.5 ± 0.1
Average pupae	Experiment	513	18.5

It has already been shown (5) that the population data from the study area conform to the negative binomial distribution, and in order to compare ratios a logarithmic transformation must be used. The E/F ratio is then represented by $\log(E + 1) - \log(F + 1)$ on the transformed scale, and its standard error is estimated from the variance in pupal and egg counts. Pooled estimates of these two variances on the logarithm scale were obtained from Morris (5). The following example illustrates the method of comparing ratios for two plots using transformed data.

	Plot K1, 1952	Plot K2, 1952
E/F ratio (original counts)	138	69
Mean, $\log(F + 1)$, pupae	1.0026	1.2674
Mean, $\log(E + 1)$, eggs	3.0552	3.0243
Mean, $\log(E + 1) - \log(F + 1)$	2.0526	1.7569
s^2 (pupae)	0.0365	0.0365
s^2 (eggs)	0.0518	0.0518

$$t = (2.0526 - 1.7569)/\sqrt{2[(0.0518/10) + (0.0365/10)]}$$
(where 10 = number of trees sampled per plot)
$$= 2.22 \; (t_{.05} = 1.959, \text{d.f.} = \infty)$$

The above method permits a comparison of actual increases between plots or between years on the same plot. Table V shows these two types of comparison. The data for Plot K1 illustrate the significant changes that may occur in the actual increase of a population in a severe infestation. The comparison between Plots K1 and K2 shows the general trend in the actual increase in a mature stand (K1) in relation to an adjacent immature stand (K2). The consistent negative values indicate an oviposition preference for mature trees, at least up to the point (1954) where the mature trees have become very thin. Blais (1) found a similar preference in a comparison of flowering and non-flowering trees.

TABLE V

COMPARISON OF TWO RATIOS AS A METHOD OF INDICATING TRENDS IN THE ACTUAL INCREASE OF A POPULATION

Plot K1—Comparison of ratio for 1 year with ratio of previous year				
Year	1951	1952	1953	1954
E/F ratio	81	138	62	22
$\text{Log}(E + 1) - \log(F + 1)$	1.8842	2.0526	1.7548	1.0591
Mean difference		0.1684	0.2978*	0.6957†
Plots K1, K2—Comparison of ratios for two plots differing in stand type				
Year	1951	1952	1953	1954
E/F ratio, K1	81	138	62	22
E/F ratio, K2	45	69	41	26
$\text{Log}(E + 1) - \log(F + 1)$, K1	1.8842	2.0526	1.7548	1.0591
$\text{Log}(E + 1) - \log(F + 1)$, K2	1.6107	1.7569	1.6205	1.3665
Mean difference	−0.2335	−0.2957*	−0.1344	+0.2074

*Significant within the 5% level.
†Significant within the 1% level.

Comparison between Actual Eggs and Expected Eggs

Theoretically, the actual eggs would equal the expected eggs in the absence of all adverse conditions affecting oviposition. In practice, the actual will be less than the expected eggs as the result of mating failure, loss through predation, and failure of the laying of the full complement of eggs. (These factors will be grouped under the term 'adult mortality' for easy reference.) In addition, but of greater importance, the actual may be greater or less than the expected eggs as the result of adult dispersal. It is important that adult dispersal be detected since it may have an important role in population trend. One method of detecting dispersal is to compare the actual with the expected egg population, but the test is necessarily only an approximate one. It is based on the assumption that the error associated with the estimated expected fecundity is negligible in comparison with the error in pupal and egg counts for a small number of trees. A least significant difference between actual and expected eggs on the logarithm scale can then be calculated from the estimates of variance noted previously and a '$t_{.05}$' value of 1.959. Actual egg density on the log scale is available from population data, while the expected egg density is represented by log (female pupae $\times$ expected fecundity).

(*a*) *Actual Eggs Greater than Expected Eggs*

Table VI shows a comparison between the actual and expected eggs when the actual is greater than the expected value. The tests show that dispersal into Plots G2 and G9 occurred in 1953. It should be noted that this conclusion is strengthened by the fact that the expected eggs have not been reduced by an adult mortality factor, and that other plots in the area showed inward dispersal in the same year.

(*b*) *Actual Eggs Less than Expected Eggs*

The two factors, outward dispersal and adult mortality, may be involved when the actual is less than the expected value. In this event no direct test for adult dispersal out of the population is available, since a reliable estimate

TABLE VI

COMPARISON BETWEEN ACTUAL AND EXPECTED EGGS SHOWING DISPERSAL INTO PLOTS G2 AND G9, 1953

Year	1953	1953
Plot	G2	G9
Degree of infestation	Light	Severe
Expected eggs*	17	47
Actual eggs*	360	247
Expected eggs†	1.2304	1.6721
Actual eggs†	2.4288	2.3645
Difference	1.1984§	0.6924§
Least significant difference ($t_{.05}$)	0.1841	0.1841

*Original counts.
†Transformed data.
§Significant within 5% level.

TABLE VII

COMPARISON BETWEEN ACTUAL AND EXPECTED EGGS WHEN THE ACTUAL IS LESS THAN THE EXPECTED VALUE

Year	1952	1954
Plot	G9	K1
Expected eggs*	2572	1078
Actual eggs*	2176	240
Per cent difference	15	78
Expected eggs†	3.4103	3.0326
Actual eggs†	3.2914	2.1873
Difference	0.1189	0.8453§

*Original counts.
†Transformed data.
§Significant within 5% level.

of adult mortality has not yet been obtained. Nevertheless, if it is assumed that the percentage of adult mortality is of a low magnitude, some indication of outward dispersal can be obtained from a comparison of actual and expected eggs as in the above example. Tentative conclusions may also be supported by the replication of data from different plots in similar stands in any one year, and also by moth catches in light traps[4] and the direct observation of moth activity. Table VII shows this comparison for two plots. On Plot G9, where the difference between actual and expected eggs is not significant, the occurrence of dispersal is not indicated. But on K1, it is doubtful whether adult mortality alone would account for the 78% difference between the actual and expected eggs. The conclusion that outward dispersal did occur in this case seems to be justified.

The hypothesis that adult mortality is of a low magnitude is partially supported from two sources. Table VIII shows the relationship of E/F ratios and expected fecundities for plots having no significant inward dispersal of moths. In light infestations, where the range in expected fecundities is small, the E/F ratios are grouped into two classes above and below 100. (This is an arbitrary grouping based on the fact that the fecundity of caged females ranges from 100 to 300 eggs, and that a similar range in E/F ratios could occur in the absence of dispersal.) If the difference between the two classes was primarily the result of dispersal, then 140 could be accepted as the mean E/F ratio for plots where no dispersal occurred. This would indicate that the adult mortality factor (196 − 140) probably does not exceed 29%.

In severe infestations, where the range in expected fecundities is much larger, the per cent differences between the expected fecundity and E/F ratio were calculated for individual plots. The differences range from 14 to 77%, and apparently fall into two classes. Of a total of 16 measurements, five range below 30%, eight above 50%, and only three have intermediate values.

[4]D. O. Greenbank, Green River Annual Reports 1949–1954.

TABLE VIII

TRENDS IN E/F RATIOS IN RELATION TO EXPECTED FECUNDITIES FOR PLOTS APPARENTLY UNAFFECTED BY MOTH DISPERSAL INTO THE POPULATION

Light infestations:	
No. of plots	23
Range in expected fecundity	169–196
Range in E/F ratios	52–178
Mean of ratios greater than 100	$140 \pm 26 (N = 11)$
Mean of ratios less than 100	$78 \pm 17 (N = 12)$
Severe infestations:	
No. of plots	16
Range in expected fecundity	58–157
Range in E/F ratios	22–138
Differences between expected fecundity and E/F ratios expressed as percentages:	
Range	14–77
Mean of per cent differences less than 30	$20 (N = 5)$
Mean of per cent differences greater than 50	$62 (N = 8)$

These data are taken to indicate that the adult mortality factor probably does not exceed 30%. Additional data on E/F ratios may provide a precise estimate of adult mortality, but, until such a figure is obtained, the occurrence of dispersal out of a population must be based on theoretical evidence.

Discussion

The errors involved in the indiscriminate use of the regression of fecundity on an index of pupal or adult size for prediction purposes outside of the population on which it is based have been discussed by Prebble (7). The results of this study conform with his conclusions and illustrate the necessity of obtaining either repeated experimental checks on existing regression equations or establishing new ones to fit existing conditions. This is the principal limitation in the technique. Further, the present study is confined to one watershed and one host species, balsam fir, and the danger of applying results to other regions is fully realized. However, if the aim of the technique is to estimate the fecundity of a population, having established the regression equation for a population with a similar infestation history, it can be a useful tool. This is particularly true when life tables are maintained on a number of 'populations' within known infestation classes, and time does not permit the measurement of fecundity by oviposition experiments for all plots each year. Another advantage is that, in the absence of severe starvation, the 'normal' regression equation will provide a means of estimating fecundity when a population declines to the endemic level.

The expected egg population can be estimated and compared with the actual egg population when the expected fecundity is known. This comparison, although not a precise test, is used to indicate the presence or absence of adult dispersal, and tentative conclusions may be drawn when the results for one plot are repeated on other plots in the area, and also when moth catches

in light traps show unusual moth activity. The detection of dispersal is important, since the budworm is subject to both local and widespread transport, and this factor may play an important role in population trend.

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OBSERVATIONS ON THE LIFE CYCLES OF DIPLOTRIAENOIDES TRANSLUCIDUS ANDERSON AND MEMBERS OF THE GENUS DIPLOTRIAENA[1]

ROY C. ANDERSON

Abstract

Diplotriaenoides translucidus Anderson, 1956 from *Seiurus aurocapillus* L. and various species of *Diplotriaena* from *Quiscalus quiscula* (L.), *Vermivora ruficapilla* (Wilson), and *Agelaius phoeniceus* (L.) were found in the air sacs of their hosts. The eggs of *D. translucidus* and the *Diplotriaena* sp. from the grackle were found in the lungs and contents of the alimentary canal. It seems most likely that the eggs of all members of the subfamily Diplotriaeninae leave the definitive host via the lungs and faeces. Eggs of *D. translucidus* were fed to a variety of invertebrates. Spirurid larvae, found in the body cavities of grasshoppers (*Camnula pellucida* (Scudder)) that were fed eggs, are considered to belong to *D. translucidus;* these larvae are described. No spirurid larvae were found in ground beetles, field crickets, tenebrionids, camel crickets, ants, or land snails that were fed eggs. Similarly no spirurid larvae were found in blowflies, dung beetles, hister beetles, or earthworms that were fed eggs of the *Diplotriaena* sp. from *Q. quiscula.*

Introduction

In 1935 Wehr (6) proposed that those filarioid worms which produce thick-shelled eggs with differentiated larvae be retained in the family Filariidae and those which produce microfilariae be placed in the family Dipetalonematidae. Life history studies on filarioids have been confined largely to the dipetalonematids. Losev, Erokhin, and Nikanorov (4) and Baumann (1) have shown, however, that *Parafilaria multipapillosa* (Condamine and Drouilly, 1878) of horses releases its eggs through a break in the skin. Muscoid flies ingest the eggs and serve as intermediate hosts. The filariid subfamily Diplotriaeninae has been a puzzling group because these worms have generally been considered to occur in the body cavities of birds where there would be no direct route for the eggs to get to the outside. However, several species of Diplotriaeninae have been reported from the air sacs (5). Chabaud (2), in addition to finding *Diplotriaena monticelliana* (Stossich, 1890) in the air sacs, notes that the eggs pass through the lungs up the trachea and out in the faeces. General observations on several species of Diplotriaeninae are reported herein as well as evidence that grasshoppers are suitable intermediate hosts of *D. translucidus* Anderson, 1956 of the ovenbird (*S. aurocapillus* L.).

Material and Methods

The birds in which members of *Diplotriaenoides* and *Diplotriaena* were found in 1956 in Algonquin Park were caught in traps and their organs were undisturbed at the time of dissection. Seven female and five male *D. translucidus* were obtained from an adult male ovenbird (*S. aurocapillus* L.)

[1]Manuscript received October 5, 1956.
Contribution from the Department of Parasitology, Ontario Research Foundation, Toronto, Ontario.

collected on June 5. Seventeen female and 23 male *Diplotriaena* sp.* were obtained from a grackle (*Q. quiscula* (L.)) captured on May 25; this bird died in captivity on May 28. Two male *Diplotriaena* sp. were collected from a Nashville warbler (*V. ruficapilla* (Wilson)) on June 8 and one female *Diplotriaena* sp. was taken from a red-winged blackbird (*A. phoeniceus* (L.)) on June 28.

The lungs, trachea, and the contents of the alimentary tract of infected birds were examined for eggs. The lungs were removed and washed thoroughly. Part of one lung was dissected in water to see if eggs were present. Portions of the alimentary tract were clamped off and removed. After the outside of the gut was thoroughly washed under running water a sample of the gut contents was removed and examined for eggs.

Eggs of *D. translucidus* and of *Diplotriaena* sp. from the grackle were removed from the vagina and anterior parts of the uteri of female worms and placed in water. These eggs were mixed with the food of various invertebrates in the following way:

1. Snails—*Zonotoides arboreus* (Say) and *Discus cronkhitei* (Newcombe) were placed in dishes with pieces of moist wood. A heavy concentration of the eggs in water was pipetted onto the wood.
2. Hister and dung beetles—A solution of water and eggs was mixed with deer faeces and placed on sand in dishes containing the beetles.
3. Blowfly larvae—The eggs were pipetted onto moist meat on which blowfly maggots were feeding. The adult flies were examined for larvae.
4. Earthworms—Eggs were mixed with earth containing the earthworms.
5. Ground beetles, camel crickets, and ants—Eggs were pipetted onto bread or white flour that was used as food.
6. Grasshoppers and crickets—Eggs were mixed with flour and the paste was spread onto clean blades of green grass.

The various invertebrates that were fed eggs were examined for the presence of larval stages. Larvae removed from them were placed in physiological saline and studied while they were still alive as well as after they had been killed and preserved.

Results

The worms in the grackle, ovenbird, Nashville warbler, and red-winged blackbird were found in the abdominal and thoracic air sacs. Some worms in the grackle and the ovenbird were projecting into the openings leading from the lungs to the air sacs. Eggs were found in the lungs and in the contents of the alimentary canals of these birds. No eggs were found in the Nashville warbler and the red-winged blackbird presumably because only worms of a single sex were found in these birds. The eggs from *D. translucidus* and *Diplotriaena* sp. from the grackle were put in water and placed in the refrigerator. The larvae were never observed to move within the eggs. Larvae

*The genus *Diplotriaena*, consisting of a great many poorly defined species, is badly in need of revision and the author is reluctant to give a specific determination of the species collected until such a revision has been made.

straightened out when pressed from eggs but no other movement was noted. The larvae in the eggs lost their smooth contours and their faintly granular appearance about three weeks after being placed in water; large vacuoles appeared in the larvae and it was assumed that they were not viable at this time.

Eggs of *Diplotriaena* sp. from the grackle were fed to 13 unidentified blowfly maggots, 25 dung beetles of the genus *Onthophagus*, nine hister beetles (Histeridae), and eight earthworms. These invertebrates were kept in captivity for 24–30 days before they were dissected. No spirurid larvae were found in any of them. Viable eggs were noted in the alimentary canals of dung beetles 6 days after they were fed eggs. Possibly the eggs failed to hatch in these invertebrates.

Thirty grasshoppers (*C. pellucida* (Scudder)) were fed eggs of *D. translucidus* and larval spirurids were subsequently found in them. These grasshoppers were dissected at various intervals after they were fed eggs (Table I). During the first few days of these experiments only grasshoppers that had died in captivity were dissected. If some of these grasshoppers had failed to feed it might account for the fact that no larvae or eggs were found in some grass-

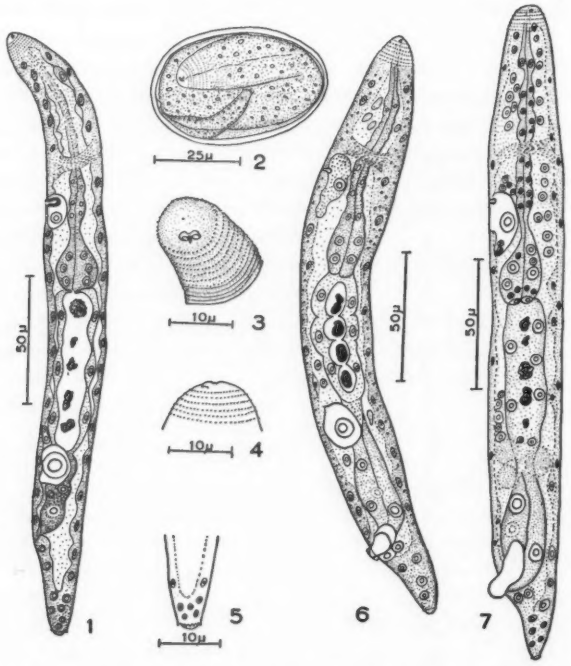
TABLE I

RESULTS OF DISSECTING GRASSHOPPERS FED EGGS OF *Diplotriaenoides translucidus*

Date fed	Date dissected	No. days	No. grasshoppers	Results
July 16	July 18	2	1	Two first-stage larvae (Fig. 1)
July 10	July 13	3	3	No larvae found
July 10	July 14	4	1	Eggs in crop and intestine
July 10	July 15	5	2	Eggs in intestine of one; other with 15 first-stage larvae
July 16	July 21	5	3	One to two first-stage larvae in each
July 16	July 21	5	4	Two with numerous first-stage larvae; no larvae found in two
July 16	July 22	6	1	No larvae found
July 10	July 17	7	2	No larvae found
July 17	July 24	7	1	No larvae found
July 16	July 26	10	1	Two first-stage larvae with anal plugs (Fig. 6)
July 17	July 28	11	1	Two first-stage larvae with anal plugs
July 16	July 31	15	1	Six first-stage larvae (Fig. 7)
July 10	Aug. 3	24	1	Three molting first-stage larvae (Fig. 8)
July 10	Aug. 6	27	1	Two degenerate first-stage larvae, one molting second-stage larva (Fig. 9)
July 10	Aug. 10	31	1	Three third-stage larvae (Fig. 14)
July 10	Aug. 13	34	1	Two third-stage larvae
July 10	Aug. 13	34	1	Four third-stage larvae in head, three in thorax, four in abdomen
July 10	Aug. 19	40	1	Three third-stage larvae in head, one in thorax, three in abdomen (four encysted)
July 10	Aug. 19	40	1	One third-stage larva in head, two in abdomen, five in thorax (four encysted)
July 10	Aug. 19	40	1	Sixteen third-stage larvae in head, six in thorax, six in abdomen (11 encapsulated)
July 16	Aug. 20	41	1	One third-stage larva in head; one in abdomen (both encysted)

hoppers examined less than a week after they were offered eggs. The grasshoppers were kept at outdoor temperatures up to July 26 after which they were placed each night in an incubator kept at 25° C. The part of the alimentary tract in which the eggs hatched was not determined. The larvae developed in the body cavity.

The first-stage larvae (Fig. 1), newly hatched from eggs fed to grasshoppers, were exceedingly active, unlike the larvae pressed from the eggs under cover slips. The anterior end was alternately protruded and retracted. The oral opening was minute. A prominent hook with a broad base was present on the dorsal side of the anterior end (Figs. 3–4); this structure may be an egg tooth involved in liberating the larva from its thick-shelled egg (Fig. 2). About seven rows of tiny, posteriorly directed spines surrounded the anterior extremity. A circle of about 14 minute spines encircled the tip of the tail (Fig. 5). The first-stage larva was about 250 μ long and had a maximum diameter of about 24 μ. The esophagus was 106 μ long; the nerve ring encircled the esophagus 50 μ from the anterior end. The anterior part of



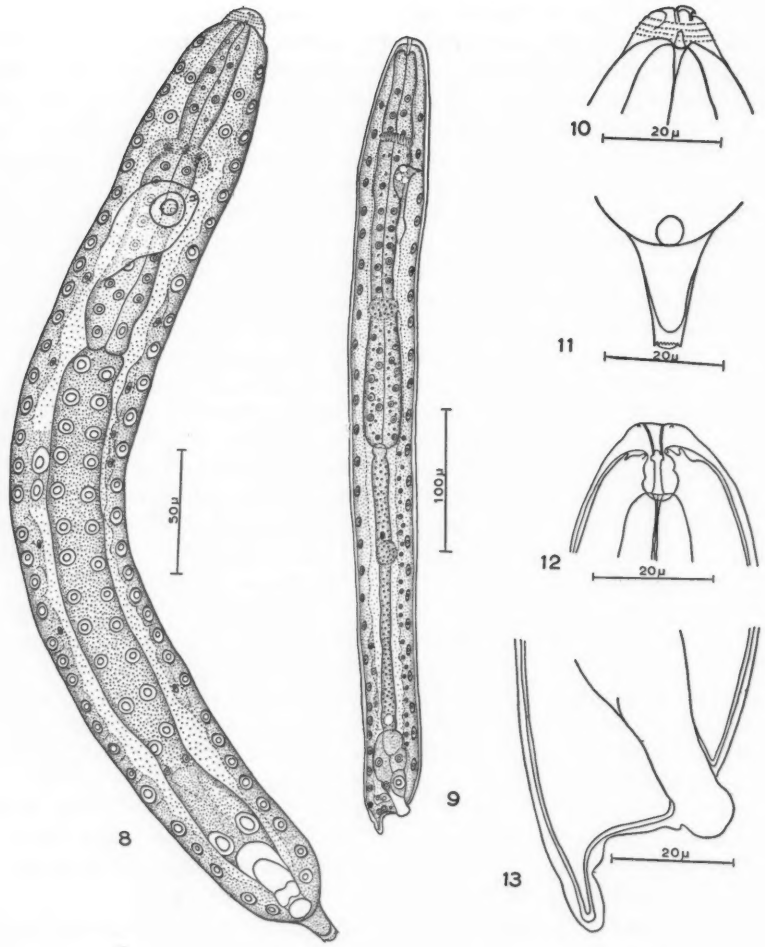
FIGS. 1–7. Larval stages of *Diplotriaenoides translucidus* from grasshoppers. Fig. 1. First-stage larva which had just hatched from egg, lateral view. Fig. 2. First-stage larva in egg. Fig. 3. First-stage larva, *en face* view showing dorsal tooth. Fig. 4. Anterior end first-stage larva, lateral view. Fig. 5. Tail end first-stage larva, lateral view. Fig. 6. First-stage larva found in grasshopper 10 days after it was fed eggs, lateral view. Fig. 7. First-stage larva found in grasshopper 15 days after it was fed eggs, lateral view.

the esophagus was narrow, whereas the posterior part was broad and almost bulb-like; at least six large nuclei were present in the posterior enlargement of the esophagus. The excretory pore lay on a tiny protuberance about 70 μ from the anterior end and it opened into a clearly defined excretory vesicle. The nucleus of the excretory cell (sinus cell?) was prominent. The intestine, 64 μ in length, was composed of three pairs of attenuated, spindle-shaped cells. Three to five masses of refractory material (yolk?) were present in the lumen of the intestine. The rectum was about 37 μ long and it seemed to be composed of only about four cells. A large cell, lying just behind the junction of the rectum and the intestine, about 67 μ from the tip of the tail, was interpreted as the genital primordium. The minute anus was about 40 μ from the tip of the tail. The cuticle was finely striated transversely and it was lined by a single row of attenuated cells. Other poorly defined cells were present between these cells and the alimentary tract.

The first indications of development were a slight thickening of the body, the appearance of an anal plug, an increase in the length of the rectum, a thickening of the intestinal cells, and the appearance of a lumen in the rectum (Fig. 6). Somewhat later the esophagus increased slightly in length and the lumen of the intestine disappeared although the refractory bodies could still be observed in the intestine (Fig. 7). In addition, the anal plug and the lumen of the rectum further increased in size. About this time various attenuated cells could be observed adhering to the cells lining the cuticle. Besides the excretory cell mentioned earlier, other smaller cells were associated with the excretory system as indicated in Fig. 7. Numerous other cells were present in the pseudocoelome.

The first-stage larva prepared to molt when it was about 400 μ in length (Fig. 8). Such a larva had the following dimensions: length 408 μ; maximum width 45 μ; nerve ring 60 μ from the anterior extremity; excretory pore 81 μ from the anterior extremity; esophagus 146 μ in length; anus 13 μ from the caudal extremity. The number of cells lining the cuticle and the number of cells composing the intestine had increased. The lumen of the rectum had increased in size. The anal plug was prominent. Much of the body of the larva behind the anus did not increase in thickness with the rest of the body and the tail was short and relatively narrow at this time. The cuticle of the first-stage larva was loose at both extremities and the various spines as well as the egg tooth could be observed easily (Figs. 10–11).

No larval stages between the molting first-stage and the molting second stage were studied since the author was reluctant to kill the grasshoppers before third-stage larvae would be expected. Nevertheless a molting second-stage larva was obtained from a grasshopper dissected 27 days after it was fed eggs of *D. translucidus* (Fig. 9). This larva had the following dimensions: length 615 μ; maximum width 52 μ; length esophagus 310 μ; nerve ring 70 μ from anterior end; excretory pore 98 μ from anterior end; length intestine 224 μ; anus 17 μ from tail end. The short, narrow tail was displaced dorsally (Fig. 13). The intestine was narrow and granular; no nuclei were



FIGS. 8–13. Larval stages of *Diplotriaenoides translucidus* from grasshoppers. Fig. 8. Molting first-stage larva found in grasshopper 24 days after it was fed eggs, ventral view. Fig. 9. Molting second-stage larva found in grasshopper 27 days after it was fed eggs, lateral view. Fig. 10. Anterior end molting first-stage larva, lateral view. Fig. 11. Tail end molting first-stage larva, ventral view. Fig. 12. Anterior end molting second-stage larva, lateral view. Fig. 13. Tail end molting second-stage larva, lateral view.

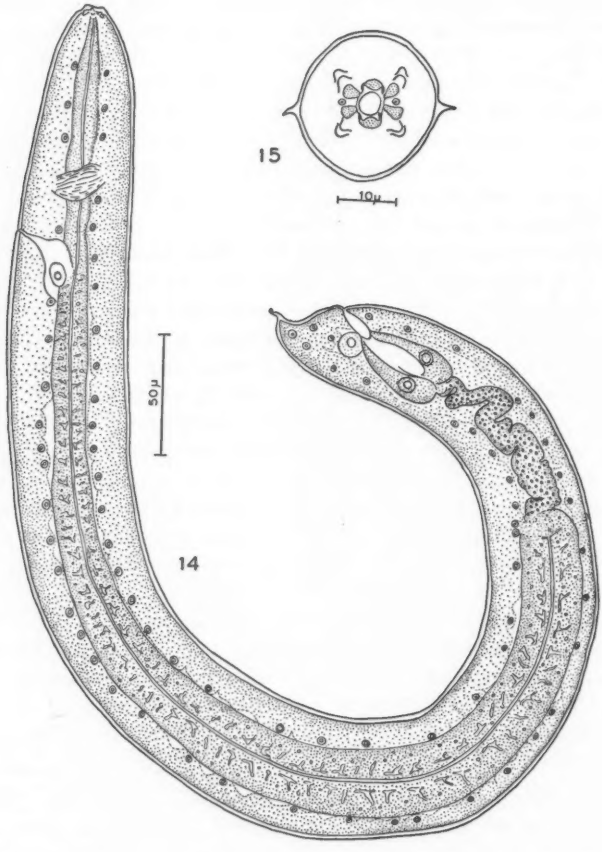
observed in the intestine. The cuticle of the larva was lined, at least on the ventral side, by a double row of nuclei. The outermost nuclei were larger and it is believed that these cells finally form the hypodermis of the third-stage larva. The innermost layer of cells perhaps form the muscle wall of the third-stage larva. A small granular body, believed to be the genital primordium, was present in the pseudocoelome 217 μ from the tip of the tail. The cuticle was loose at the extremities. The characteristics of the tail of the third-stage larva could be observed beneath the cuticle of the second stage. The buccal capsule was broad and finger-like projections could be seen surrounding the oral opening (Fig. 12). The lining of both the esophagus and rectum are evidently shed at this stage.

Larvae, regarded as third-stage larvae of *D. translucidus*, were found 31 days after eggs were fed to the grasshoppers (Fig. 14). As no further development was noted in larvae removed from grasshoppers that were kept for an additional 10 days it was concluded that all these larvae were fully developed. Most of the larvae were free in the body cavity but some were found in small cysts; in one instance two larvae were found in a single cyst. Encysted larvae were found only after 40 days, suggesting that encystment takes place some time after the larvae have attained the third stage. Larvae were found in the head, thorax, and abdomen. The third-stage larva had the following dimensions: length 710 μ; maximum width 49 μ; length esophagus 560 μ; length of rectum 49 μ; nerve ring 65 μ from the anterior end; excretory pore 90 μ from the anterior end; anus 25 μ from the tail end. The larva was short and stout and after fixation it usually assumed the shape of a horseshoe. Lateral alae extended nearly the whole length of the body. The anterior part of the esophagus was narrow. The remainder of the esophagus was extremely broad, especially posteriorly, and it was packed with granules. The narrow, granular, convoluted intestine occupied a short region between the esophagus and intestine. The convoluted condition of the intestine and the great length of the esophagus as compared to that found in the molting second-stage larva suggests that a rapid backward growth of the esophagus, without a proportional increase in body length, took place after the second molt. The rectum was composed of four large cells which tapered towards the minute anus; an elongate lumen was present between these cells. The genital primordium was not located with certainty; presumably it is difficult to see since the esophagus and intestine fill the pseudocoelome almost completely. The nucleus of the excretory cell was prominent but other details of the excretory system were not determined although it was noted that the prominent excretory cell passed into other structures posteriorly, possibly gland cells and lateral canals.

The anterior end of the third-stage larva was minute and complex and great difficulty was experienced in determining the details of its structure. The following description, based primarily on *en face* preparations that were studied with oil immersion lens, must therefore be regarded as tentative. The oral opening was more or less circular and it was surrounded by a thickened

cuticular ring (Fig. 15). This ring gave off four prominent protuberances, two on the dorsal side and two on the ventral side. Six lip-like structures were present, those on the mediolateral sides being the most clearly defined. The lip-like structures on the dorsal and ventral sides were broader and sometimes seemed to be bilobed anteriorly but this point was not settled. Minute structures identified as amphids were observed between the mediolateral lip-like structures, posterior to which were four pairs of prominent submedian papillae. The most anterior papilla was largest and it generally obscured the papilla behind it.

The grasshoppers used in these studies were captured in an open field in Algonquin Park and one naturally wonders if perhaps they might have been



FIGS. 14–15. Larval stages of *Diplotriaenoides translucidus* from grasshoppers. Fig. 14. Third-stage larva, lateral view. Fig. 15. Anterior end third-stage larva, *en face* view.

infected prior to their capture and if perhaps the larvae found in these grasshoppers belonged to some spirurid other than *D. translucidus*. Several considerations support the view that these larvae resulted from the experimental feeding of the eggs of *D. translucidus*.

1. No larvae were found in 10 grasshoppers collected on June 11, seven on June 13–14, and 20 on Aug. 19 from the same locality as the experimental grasshoppers; the grasshoppers examined on Aug. 19 were the same size and presumably the same age as the experimental grasshoppers.

2. The experimental grasshoppers were perhaps only a week or 10 days old at the outset of the experiment and thus had only a limited time in which to acquire natural infections. In addition the grasshoppers were collected at random over a large area.

3. The series of larvae from the first stage in the eggs to the molting first stage can undoubtedly be regarded as belonging to *D. translucidus* inasmuch as the first-stage cuticle, with its characteristic spines, was present throughout. In addition subsequent larval stages found in the grasshoppers seem to follow the first-stage larva morphologically.

4. All seven of the grasshoppers examined 31 days after eggs were fed to them contained third-stage larvae; this incidence is probably too high for the infections to have been acquired naturally. In addition 21 of the 30 grasshoppers used in these experiments contained either eggs or larval stages. It has already been noted that many of the uninfected grasshoppers died during the first few days of the experiments possibly because they failed to feed.

Unfortunately an ovenbird was not available to which to feed third-stage larvae. Therefore 36 larvae were given orally to a white-crowned sparrow, (*Zonotrichia albicollis* (Gmelin)), but no nematodes were found in this bird when it was autopsied 26 days later.

Eggs of *D. translucidus* were fed to nine ants (*Formica* sp.), 18 assorted ground beetles (Carabidae) of the genera *Harpalus*, *Platynus*, and *Euferonia*, eight *Zonotoides arboreus* (Say), seven *Discus cronkhitei* (Newcombe), two tenebrionids (Tenebrionidae), four camel crickets (Rhaphidophorinae), and six field crickets (Gryllidae) of the genera *Memobius* and *Acheta* but there was no evidence that the eggs hatched in these invertebrates and no spirurid larvae were found in them when they were examined 3 to 4 weeks after being fed eggs.

Discussion

There is no evidence that grasshoppers are the natural intermediate hosts of *D. translucidus*. On the contrary, food habit studies of this bird carried out by the members of the Ontario Department of Lands and Forests indicate that ovenbirds rarely, if ever, eat grasshoppers. Probably, therefore, some other insect serves as an intermediate host in nature. Grasshoppers could conceivably be the natural intermediate hosts of *Diplotriaena* spp. that occur in Corvidae as these birds eat grasshoppers and their faeces frequently contaminate the vegetation in open areas where grasshoppers occur.

Probably various other members of the family Filariidae live in the air sacs of birds and their eggs pass through the lungs as has been shown to occur in *D. translucidus*. Species of *Dicheilonema* Diesing 1861, *Serratospiculum* Skrjabin, 1915, as well as *Hamatospiculum accipitris* Yamaguti, 1941, *Monopetalonema alcedinis* (Rudolphi, 1819), *M. angustispiculum* (Sandground, 1933), *Pseudaprocta decorata* Li 1933, *Lissonema yaba* (Connal, 1912), and *Tetracheilonema tringae* (Adams, 1933) all produce thick-shelled eggs although they are usually regarded as occurring in the body cavities of birds. However, *Dicheilonema ciconiae* (Gmelin, 1791), *Serratospiculum turkestanicum* Skrjabin, 1915, and *S. guttatum* (Schneider, 1866) are reported from the air sacs of birds (5). The author suggests the possibility, therefore, that all these species live in air sacs and that they have life cycles similar to that indicated for *D. translucidus*. Since reptiles, like birds, possess air sacs it is highly probable that species of *Hastospiculum* Skrjabin, 1923 likewise occur in air sacs even though they are reported to occur under the peritoneum (3). Significantly no oviparous member of the family Filariidae is known from the body cavities of mammals where there would be no possibility of confusing the peritoneum with air sacs (*Papillosetaria* Vevers, 1922 and *Setaria* Viborg, 1793, although at present placed in the Filariidae, produce microfilariae). Thus in the subfamily Diplotriaeninae and possibly in the other genera and species mentioned above, we have life cycles which are evidently similar to those found in the spiruroids although the adult worms occur in the respiratory tract rather than the alimentary canal.

Acknowledgments

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TAXONOMIC STUDIES ON THE GENERA APROCTELLA CRAM, 1931 AND CARINEMA PEREIRA AND VAZ, 1933 WITH A PROPOSAL FOR A NEW GENUS PSEUDAPROCTELLA N. GEN.[1]

ROY C. ANDERSON

Abstract

Aproctella stoddardi Cram, 1931, collected from *Zonotrichia albicollis*, *Geothlypis trichas*, *Hylocichla ustulata*, and *Bonasa umbellus*, is redescribed. The genus *Aproctella* is emended and two species are recognized in this genus, namely, *A. stoddardi* Cram, 1931 (genotype) and *A. carinii* (Pereira and Vaz, 1933) n. comb. The genus *Carinema* Pereira and Vaz, 1933 is placed as a synonym of *Aproctella*. A new genus *Pseudaproctella* is proposed to have as genotype *P. nuda* (Hamann, 1940) n. gen. n. comb. and to include *P. dubia* (Johnston and Mawson, 1940) n. gen. n. comb. Specimens described by YBarra (1948) as *Aproctella nuda* Hamann, 1940 are regarded as belonging to *A. stoddardi*. *Microfilaria fallisi* Brinkmann, 1950 is regarded as probably a synonym of *A. stoddardi* and the new combination *Paraprocta graucalinum* (Johnston and Mawson, 1940) n. comb. is suggested for *Carinema graucalinum*.

Introduction

Dipetalonematid nematodes, belonging to the same species, have been collected from several species of birds in Algonquin Park. These worms possess distinct, lateral protuberances of the buccal capsule. Originally the author thought these nematodes represented a new genus within the family Dipetalonematidae Wehr, 1935 since lip-like structures have not been used to distinguish any genus in this family. Further study showed, however, that similar structures are present on *Carinema carinii* Pereira and Vaz, 1933. In addition Dr. Everett E. Wehr, of the United States Department of Agriculture, kindly informed the author (in litt.) that he had noted in 1934 that *Aproctella stoddardi* Cram, 1931 possessed lip-like structures although they were not mentioned in the original descriptions by Cram (2, 3). Dr. Wehr also told the author that YBarra (8) in 1948 had described lip-like structures on specimens from Mexico identified by YBarra as *Aproctella nuda* Hamann, 1940. Moreover, the author noted the similarity of *Microfilaria fallisi* Brinkmann, 1950 to the microfilaria associated with those nematodes possessing lip-like structures found in various birds in Algonquin Park. It was necessary, therefore, to re-examine the type specimens of *A. stoddardi*, *C. carinii*, *A. nuda*, and *M. fallisi* and to compare them with the specimens collected from birds in Ontario.

In this paper the author will endeavor to show that (*i*) the specimens found in Ontario birds belong to *A. stoddardi*, (*ii*) *A. nuda* Hamann does not belong to the genus *Aproctella*, (*iii*) the specimens described as *A. nuda* by YBarra belong to *A. stoddardi*, (*iv*) the genus *Carinema* is a synonym of *Aproctella*, and *C. carinii* is a valid species in *Aproctella*, and (*v*) *M. fallisi*

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is probably a synonym of *A. stoddardi*. *A. stoddardi* is redescribed from specimens collected from birds in Ontario. In addition the generic diagnosis of *Aproctella* is emended and a new genus *Pseudaproctella* is proposed to accommodate *A. nuda* Hamann, 1940 and *Carinema dubia* Johnston and Mawson, 1940. *C. graucalinum* Johnston and Mawson, 1940 is reallocated to the genus *Paraprocta* Maplestone, 1931.

Specimens Studied

White-throated sparrow (*Zonotrichia albicollis* (Gmelin))
No. 1—1♂, 3♀♀ ; body cavity (July 25, 1953).
No. 2—1♂, – ; body cavity (Oct. 9, 1952).
No. 3—1♀, – ; body cavity (May 13, 1956).

Maryland yellow throat (*Geothlypis trichas* (L.))
2♀♀, – ; pericardial sac (July 10, 1954).

Olive-backed thrush (*Hylocichla ustulata* (Tschudi))
No. 1—2♂♂, 1♀ ; body cavity (Aug. 16, 1954).
No. 2—1♀, – ; kidney (May 16, 1956).

Ruffed grouse (*Bonasa umbellus* (L.))
1♀, – ; kidney (July 28, 1956).

In addition to the specimens from Algonquin Park listed above, the author has examined the following type specimens:

A. stoddardi Cram, 1931—U.S. National Museum Helminthological Collection Nos. 27618 and 28995 (received through the courtesy of Mr. Allan McIntosh, of the U.S. Department of Agriculture).

C. carinii Pereira and Vaz, 1933—Helminthological Collection of the Institute of Biology of São Paulo, Brazil, No. 210 (received through the courtesy of the Director of the Institute of Biology, São Paulo).

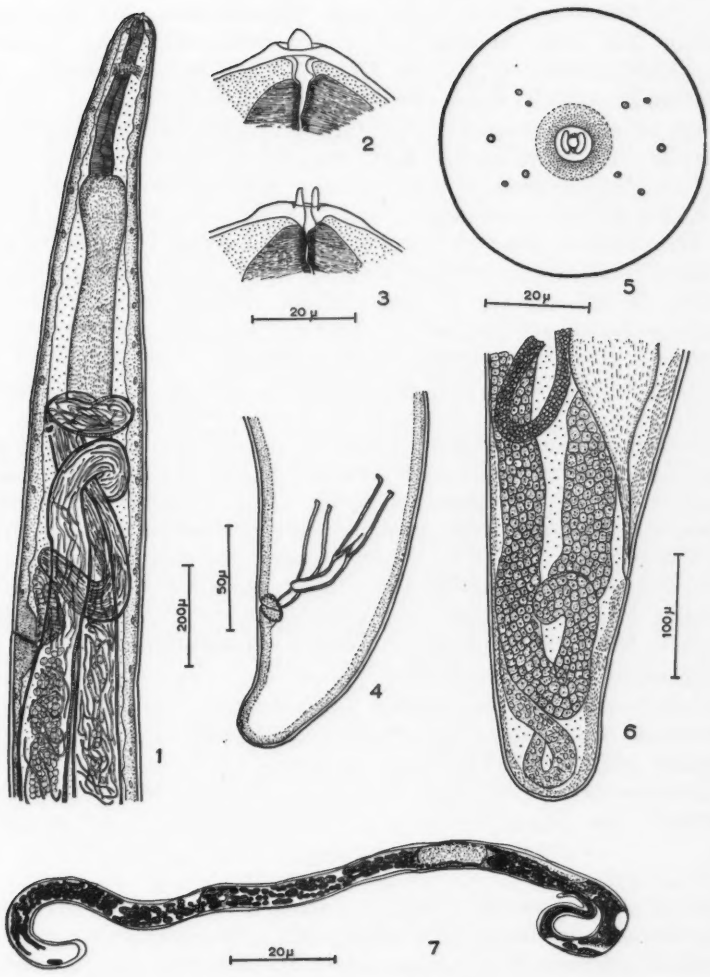
A. nuda Hamann, 1940—Helminthological Collection of Purdue University, West Lafayette, Indiana (received through the courtesy of Prof. R. M. Cable of Purdue University).

M. fallisi Brinkmann, 1950—Helminthological Collection of the Ontario Research Foundation.

Aproctella stoddardi Cram, 1931

(Figs. 1–7)

Description.—Dipetalonematidae Wehr, 1935; Splendidofilariinae Chabaud and Choquet, 1953; *Aproctella* Cram, 1931. Small worms tapering markedly at extremities. Oral opening circular, bordered by weakly cuticularized ring. Buccal capsule moderately well-developed. Lateral walls of buccal capsule bearing weakly cuticularized projections which protrude prominently from oral opening forming delicate, rounded, lip-like structures. Four pairs of tiny, submedian, cephalic papillae. Amphids lateral, slightly posterior to outermost papillae. Esophagus short, undivided. Cuticle on dorsal and ventral surfaces of body with longitudinal striations.



FIGS. 1–7. *Aproctella stoddardi* Cram, 1931.

FIG. 1. Anterior end female, lateral view.
FIG. 2. Anterior extremity of female, lateral view of stoma.
FIG. 3. Anterior extremity of female, dorsal view of stoma.
FIG. 4. Posterior end of male, lateral view.
FIG. 5. Anterior extremity of female, *en face* view.
FIG. 6. Posterior end of female, lateral view.
FIG. 7. Microfilaria, Giemsa's stain.

Male (one specimen).—Length 7.6 mm. Tail coiled once. Width of head 0.074 mm. Width of body at end of esophagus 0.10 mm.; at anus 0.060 mm. Maximum width 0.14 mm., attained near middle of body. Nerve ring 0.16 mm. from anterior end. Buccal capsule 4–5 μ in length. Esophagus 0.37 mm. Spicules stout and curved, each ending in rounded point. Right spicule 53 μ; left 73 μ. Caudal papillae absent. Anus surrounded by cuticular ring 0.067 mm. from posterior end.

Female (three specimens).—Length 14.0–16.5 mm. Width of head 0.071–0.084 mm. Width of body at nerve ring 0.11 mm.; at anus 0.13 mm. Maximum width 0.24–0.26 mm., attained near middle of body. Nerve ring 0.11–0.12 mm. from anterior end. Vulva, a transverse slit, 1.3–1.6 mm. from anterior end, not opening on protuberance. Vagina long and convoluted dividing into two opisthodelphic uteri packed with microfilarioid embryos. Buccal capsule 4–5 μ in length. Esophagus 0.33–0.37 mm. Intestine broad and granular, enlarging to maximum width of 0.069–0.10 mm. immediately behind esophagus. Rectum narrow. Anus 0.15–0.18 mm. from caudal extremity. Tail rounded with pair of tiny ventrolateral protuberances.

Microfilaria (eight specimens, Giemsa's stain).—Long and slender with delicate, transparent sheath. Anterior end rounded, with cephalic space. Posterior end tapering to moderately slender tail. Nuclear column composed of three rows of nuclei interrupted by nerve ring and diffuse inner body. Length 170–217 (av. = 193) μ. Relative positions of fixed points as percentages of the total length as follows: cephalic space 2.6–4.3 (av. = 3.2)%; nerve ring 19–26 (23)%; excretory pore 31–40 (36)%; anterior border of inner body 58–69 (63)%; posterior border of inner body 67–76 (72)%; first rectal cell 70–76 (73)%; anal pore 79–86 (83)%.

Host.—*Z. albicollis* (Gmelin), white-throated sparrow.

Location.—Abdominal cavity; microfilariae in blood.

Locality.—Algonquin Park, Ontario, Canada.

Specimens.—Helminthological Collection of the Ontario Research Foundation.

Discussion

Tables I and II show that the dimensions of the nematodes from *G. trichas*, *H. ustulata*, *Z. albicollis* (Nos. 2 and 3), and *B. umbellus* agree closely with those described above from *Z. albicollis* (No. 1). No morphological differences could be found to separate these specimens and it is concluded that they are conspecific. These specimens agree in all particulars with the type specimens of *A. stoddardi*. These latter specimens have the lip-like structures found in the specimens from birds from Algonquin Park. Only one of these structures is present on the female type specimen. However, the author has a specimen of *A. stoddardi* from *H. ustulata* (No. 2) which has only one of these protuberances and he concludes that this specimen, as well as the type of *A. stoddardi*, is abnormal in this respect. Moreover, the anus of the female

TABLE I

COMPARISONS OF MALE SPECIMENS OF *Aproctella stoddardi* CRAM, 1931 FROM BIRDS IN ONTARIO

(MEASUREMENTS IN MILLIMETERS)

	Host		
	Zonotrichia albicollis No. 1	*Zonotrichia albicollis* No. 2	*Hylocichla ustulata*
Length	7.6	6.8	7.6, 6.5
Esophagus	0.37	—	0.34, 0.34
Nerve ring	0.16	—	0.14, 0.16
Tail length	0.066	0.070	0.087, 0.061
Left spicule	0.073	0.074	0.092, 0.071
Right spicule	0.053	0.064	0.061, 0.063

TABLE II

COMPARISON OF MALE SPECIMENS OF *Aproctella stoddardi* CRAM, 1931 FROM BIRDS IN ONTARIO

(MEASUREMENTS IN MILLIMETERS)

	Host					
	Zonotrichia albicollis No. 1	*Zonotrichia albicollis* No. 3	*Hylocichla ustulata* No. 1	*Hylocichla ustulata* No. 2	*Geothlypis trichas*	*Bonasa umbellus*
Length	14.0 -16.5	9.6	13.2	10.2	9.0, 9.7	8.1
Esophagus	0.33- 0.37	—	—	0.33	0.38, 0.30	0.31
Nerve ring	0.11- 0.12	0.13	0.14	0.12	0.13, 0.13	0.13
Tail length	0.15- 0.18	0.13	0.15	0.10	0.098, 0.12	0.11
Vulva	1.3 - 1.6	1.2	—	1.1	0.88, 0.84	0.84

type of *A. stoddardi* is 133 μ from the caudal extremity and not "atrophied or absent" as reported by Cram (2, 3).

A. nuda Hamann, 1940 was originally described from *Chaetura pelagica* (Linné) from Indiana (4). According to Hamann, *A. nuda* differs from *A. stoddardi* in the following characters: "*A. nuda* is larger and does not possess the dorsal and ventral fields of coarse cuticular striations described for *A. stoddardi;* other differences are the presence of the oral ring and the dissimilar spicules in *A. nuda*". According to YBarra (8), who redescribes worms identified as *A. nuda* from *Phainopopla nitens* from Mexico, *A. nuda* " . . . es muy semejante a *Aproctella stoddardi* Cram, 1931, pero difiere de ella en el tamaño, pues *A. stoddardi* es mayor y *A. nuda* posee un pequeño anillo quitenoso rodeando la abertura bucal y presenta engrosamientos cuticulares dorsales y ventrales". A re-examination of the type specimens of *A. nuda* shows, however, that YBarra redescribed a species other than *A. nuda*. *A. nuda* does not possess the buccal protuberances, found in *A. stoddardi* and in the specimens described from Mexico by YBarra (see Figs. 8–9). In addition the dorsal and ventral fields of the cuticle of *A. nuda* are devoid of longitudinal cuticular striations. These differences are sufficient to show that *A. nuda* does not belong in the genus *Aproctella*. The specimens described by YBarra clearly belong to *A. stoddardi* as there are no size or morphological differences which would serve to distinguish them from Cram's

specimens. The small chitinous ring mentioned by YBarra does not refer to the same structure in *A. nuda* Hamann and moreover YBarra is incorrect in implying that ventral and dorsal striations of the cuticle are absent on *A. stoddardi.* Thus the author relegates YBarra's *A. nuda* to the synonymy of *A. stoddardi. A. nuda* Hamann will be reallocated later in this paper.

Unfortunately the type specimens of *C. carinii* Pereira and Vaz, 1933 from *Xanthornus* sp. are disintegrated and none of the observations made by Pereira and Vaz, 1933 could be confirmed. Nevertheless their description of *C. carinii* is sufficient to show that *Carinema* is a synonym of *Aproctella.*

In their description of *C. carinii,* Pereira and Vaz state that "bocca circumdada de 2 papillas conicas ponteagudas, dando entrada a um pequeno vestibulo curto e estreito não chitinisado. Ainda na extremidade cephalica apparecem 2 pares de pequenas papillas sub-medianas" (7). This statement shows that Pereira and Vaz distinguished between the two lateral projections of the walls of the buccal capsule, which they referred to as "papillas conicas ponteagudas", and the cephalic papillae. Their illustration of the head end also shows these cuticular projections although it suggests that they originate beside the oral opening instead of from the lateral walls of the buccal capsule. Apparently the buccal projections found in *C. carinii* have not been used to distinguish *Carinema* from other genera because (*a*) they were not stressed by Pereira and Vaz, who separated their new genus from *Chandlerella* by the presence of cephalic papillae, the position of the vulva, and the anterior direction of the vagina and (*b*) because in their generic diagnosis they state only that the "bocca circumdada de 2 pequenas papillas conicas; papillas cephalicas presentes" and in the English summary at the end of their paper that the mouth is " . . . simple without lips, surrounded by two very small head papillae; with a pair of submedian cephalic papillae". Thus the unusual buccal projections, which are the most interesting features of *C. carinii,* have either been overlooked by authors reading the description of Pereira and Vaz or incorrectly interpreted as cephalic papillae.

Since *C. carinii* possesses the same buccal protuberances found in *Aproctella* there is no alternative but to place *Carinema* as a synonym of *Aproctella.* It is assumed that future study will show that *C. carinii* possesses the dorsal and ventral striations of the cuticle found in *A. stoddardi. A. carinii* (Pereira and Vaz) n. comb. is very similar to *A. stoddardi* and will perhaps later be regarded as a synonym of this species. However, there are certain described differences between the two species that make it advisable to keep them separate at the present time. *A. carinii* is a somewhat larger species than *A. stoddardi* and the male possesses two tiny, caudal papillae which have not been observed on *A. stoddardi.*

M. fallisi Brinkmann, 1950 was described from ruffed grouse collected in the central part of Ontario, including Algonquin Park (1). The author has repeatedly examined infected grouse from this part of Ontario but he was unable to find the adult worms. It was noted, however, that *M. fallisi* was extremely similar to the microfilariae found in passerine birds that were

infected with *A. stoddardi.* Brinkmann, however, failed to locate a sheath on *M. fallisi* whereas one is present on the microfilaria from passerine birds infected with *A. stoddardi.* A re-examination of the type specimens of *M. fallisi* has revealed, however, that this microfilaria possesses a delicate, transparent sheath and is, in fact, indistinguishable from the microfilariae of *A. stoddardi* in passerine birds in Algonquin Park. In 1956 Mr. Newton Kingston, of the University of Toronto, collected a mature female *A. stoddardi* from the kidney of an infected grouse from Algonquin Park. Microfilariae similar to the microfilaria of *A. stoddardi* were found in the blood. It is concluded, therefore, that *M. fallisi* is probably a synonym of *A. stoddardi. A. stoddardi* was reported by Cram to occur in the body cavities of ruffed grouse in the eastern United States. This species, however, may occur in various sites in the definitive host as we have taken specimens from the kidney and pericardial sac as well as the body cavity of passerine birds. The adults of *M. fallisi* were presumably not located earlier in grouse in Ontario because they are exceedingly small and occur in small numbers in various tissues and tissue spaces of the host.

The author, therefore, recognizes the following species in the genus *Aproctella:*

1. *A. stoddardi* Cram, 1931
   Syn. *A. nuda* YBarra, 1948 (*nec* Hamann, 1940)
   ? *M. fallisi* Brinkmann, 1950
   Hosts.—*Colinus virginianus* (type host), *Bonasa umbellus* (L.), *Phainopopla nitens, Z. albicollis* (Gmelin), *Geothlypis trichas* (L.), *Hylocichla ustulata* (Tschudi).
   Location.—Body cavity, pericardial sac, kidney.
   Locality.—Eastern United States (type locality), Mexico, Ontario.
   Specimens.—U.S. National Museum Helminthological Collection Nos. 27618 and 28995 (types); Helminthological Collection, Institute of Biology, Mexico, No. 127–1; Helminthological Collection, Ontario Research Foundation, Toronto.
2. ***A. carinii*** (Pereira and Vaz, 1933) n. comb.
   Syn. *C. carinii* Pereira and Vaz, 1933
   Host.—*Xanthornus* sp.
   Location.—Body cavity.
   Locality.—Brazil.
   Specimens.—Helminthological Collection, Institute of Biology, São Paulo.

The author suggests the following amended diagnosis of the genus *Aproctella:*

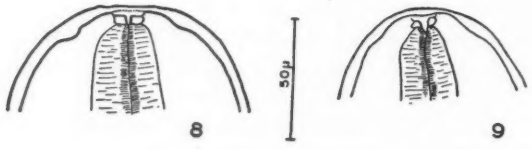
Aproctella Cram, 1931
(syn. *Carinema* Pereira and Vaz, 1933)

Dipetalonematidae Wehr, 1935; Splendidofilariinae Chabaud and Choquet, 1953. Small worms tapering markedly at extremities. Oral opening circular, bordered by weakly cuticularized ring. Buccal capsule moderately well-

developed. Lateral walls of buccal capsule bearing delicate, cuticularized, projections which protrude prominently from oral opening forming delicate rounded, lip-like structures. Four pairs of tiny, submedian papillae. Amphids lateral, slightly posterior to outermost papillae. Esophagus short, undivided. Cuticle on dorsal and ventral surfaces of body with longitudinal striations, lateral surfaces smooth. *Male:* Spicules short, subequal, and morphologically similar. *Female:* Vulva a considerable distance behind junction of esophagus and intestine. Anus patent, a considerable distance from caudal extremity.

Genotype: *A. stoddardi* Cram, 1937.

Johnston and Mawson (1940) have placed two other species in the genus *Carinema*, namely, *C. dubia* and *C. graucalinum* (5). Neither species possesses the buccal protuberances found in *Aproctella* (syn. *Carinema*) and they cannot be placed in this genus. *C. dubia*, however, has a thickened, cuticularized ring at the base of the stoma like that found in *A. nuda* Hamann, 1940 (*nec* YBarra, 1948) (Figs. 8, 9) and the writer proposes that these two species be placed together in a new genus with *A. nuda* as the genotype.



FIGS. 8–9. *Pseudaproctella nuda* (Hamann, 1940) n. gen. n. comb.
FIG. 8. Anterior end female type specimen, lateral view.
FIG. 9. Anterior end male type specimen, lateral view.

Pseudaproctella n. gen.

Dipetalonematidae Wehr, 1935; Splendidofilariinae Chabaud and Choquet, 1953. Small worms tapering markedly at extremities. Anterior end without lips or other conspicuous ornamentation. Stoma broad and shallow, the base of which contains a thickened cuticularized ring. Esophagus usually undivided. Cuticle smooth except for distinct transverse striations. *Male:* Spicules short, subequal, and morphologically similar. Caudal papillae apparently absent. *Female:* Vulva a considerable distance behind junction of esophagus and intestine. Anus patent, a considerable distance from caudal extremity.

Genotype: ***P. nuda*** (Hamann, 1940) n. gen. n. comb.
Syn. *A. nuda* Hamann, 1940 (*nec* YBarra 1948)
Host.—*Chaetura pelagica* (L.).
Location.—Body cavity.
Locality.—Indiana, U.S.A.
Type specimens.—Helminthological Collection of Purdue University, Indiana, U.S.A.

Other species: ***P. dubia*** (Johnston and Mawson, 1940) n. gen. n. comb.

Host.—*Pseudopsittacus mclennani*

Location.—Body cavity.

Locality.—North Queensland, Australia.

Type specimens.—Museum of South Australia.

There are several errors in the description of *P. dubia* (5). The anus of the male is said to be 0.7 mm. from the posterior end although an illustration indicates that it is perhaps 0.07 mm. from the posterior end. The spicules are said to be equal and 10 μ long although an illustration suggests that they are slightly subequal and about 100 μ long. The anus of the female of *P. dubia* was not observed by Johnston and Mawson and it was apparently not observed in *P. nuda* by Hamann. However, the anus is 157 μ from the caudal extremity in the female type of *P. nuda*. *P. dubia* is a larger species than *P. nuda* and the esophagus is said to be clearly divided into two parts in some specimens.

C. graucalinum Johnston and Mawson, 1940 from *Graucalus melanops* cannot be satisfactorily placed in any genus within the subfamily Splendidofilariinae but it is nevertheless more correctly allocated to *Paraprocta* Maplestone, 1931 (6) than to either *Aproctella* or *Pseudaproctella*. In *Paraprocta* the spicules are clearly subequal; one is almost twice the size of the other. In *C. graucalinum* the spicules are reported as " . . . subequal, about 60 μ long . . . " (5). The author feels, however, that this nematode should be placed provisionally in the genus *Paraprocta*. The proposed citation is ***Paraprocta graucalinum*** (Johnston and Mawson, 1940) n. comb.

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THE EMBRYOLOGY OF THE BLACK WIDOW SPIDER, LATRODECTUS MACTANS (FABR.)[1]

J. G. REMPEL

Abstract

The embryonic development of *Latrodectus mactans*, the black widow spider, from the time the egg is laid until the embryo is ready to emerge is described and an attempt made to correlate the internal developmental changes with changes in external body form. In agreement with observations of early students of spider embryology it is found that vitellocytes give rise to the mesenteron and hence the yolk cells are considered as the entoderm. The first blood cells are derived from extraembryonic cells, and are therefore ectodermal. A theory of head segmentation is suggested that contrasts with commonly accepted theories of arthropod segmentation by postulating that the spider brain is a syncerebrum made up of a protocerebrum, a greatly reduced tritocerebrum (= stomodaeal bridge), and cheliceral ganglia. The events in the development of the various systems in general closely parallel those described for many other species of spiders, but differ in some important respects from those described for another species of *Latrodectus*, namely *Latrodectus hasselti* Thorell.

Introduction

The Arachnida have been a favorite subject of invertebrate embryologists for more than a century. The ease of collecting the cocoons, the presence of large numbers of eggs of uniform age in a cocoon, and the thinness of the chorion, which makes sectioning easy and which permits observation of the embryo in live material, have been contributing factors. Recently Holm (18) has shown that spiders also form a suitable subject for studies in experimental embryology. A considerable amount of work of high quality was done on spider embryology before the close of the last century. Extended publications appeared shortly after the first decade of this century. Then followed excellent studies of the morphology, biology, and systematics of spiders. Since an extended review of the literature is found in the publications by Holm (16, 18), the writer will mention here only those publications which he consulted most extensively during the preparation of this paper.

Schimkewitsch (46) published a long paper on the embryology of *Thelyphonus caudatus*, a member of the Pedipalpida, and made extensive comparisons with the embryology of the Araneida and Phalangida. The paper is well illustrated. Wallstabe (51) studied the changes in the external body form during the embryology of *Agelena labyrinthica*. Montgomery (37) published a study of the development of *Theridium tepidariorum*. Since the latter belongs to the same family of spiders as the black widow, namely Theridiidae, a comparison of the development of the two species proved interesting and instructive. A fine contribution to our knowledge of spider embryology was made by Kautzsch (28, 29) when he published his papers on

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Contribution from the Department of Biology, University of Saskatchewan, Saskatoon, Sask. This investigation was supported financially by the National Research Council of Canada.

the development of *A. labyrinthica*. An excellent survey of the general field of spider embryology is found in Holm (16). Moreover, in the writer's opinion, Holm (18) has made the most noteworthy contribution toward an understanding of embryonic development in spiders. His work has all the attributes of a "classic". Finally, Bonnet's (6) Bibliographia Araneorum is an indispensable guide to the literature on arachnids.

In spite of the great economic importance and the almost world-wide distribution of the members of the genus *Latrodectus*, the embryology of the group has received scant attention. Deevey (12) studied the development of *Latrodectus mactans* (Fabr.) at different rates of feeding. For obvious reasons this work could be extended to the postembryonic period only. The writer has come across only one paper that deals with the embryology of these spiders. Sivickis and Filoteo (47) have published observations on the embryology of *Latrodectus hasselti* Thorell, but their paper is brief and contains apparent inaccuracies. For that reason an extended study of the developmental history of *L. mactans* seemed to be desirable. Moreover, since studies in the past have often been concentrated on internal development as revealed in sections or on external development as revealed by superficial examination, an attempt to correlate the internal developmental changes with changes in external body form promised to be rewarding.

The black widow spider is considered to be the most important spider of the Americas. The literature on the poisonous properties of this species is voluminous. The reader will find an extended review of this subject in Thorp (50), Baerg (1, 2), Blair (3), Bogen (4, 5), and D'Amour *et al.* (11). In geographic distribution the species extends from southern Canada, through the United States and Mexico, into Central America and deep into South America. In the United States it is found from the Pacific coast (15) to the Atlantic coast (26). In Canada the species has been found on Vancouver Island, in the interior of British Columbia, in southern Alberta, and in southwestern Saskatchewan (O'Rourke (39)). According to Chamberlin and Ivie (8), *L. mactans hesperus* is found in the United States and Canada west of the Rocky Mountains. The form found in Saskatchewan belongs to *hesperus*, and we assume that the other records for western Canada also represent this form. O'Rourke (39) also reports the species from southern Ontario. This is probably the eastern, or *mactans*, variety. On the dry grassy plains of southwestern Saskatchewan the species is most often found in forsaken gopher and badger holes.

Material for this study was first collected by Mr. Glenn Burgess, of the Dominion Entomology Laboratory, Saskatoon, Sask. His collection, made near the town of Govenlock in July, 1952, contained several dozen egg masses with embryos in various stages of development. In July, 1953, adult female spiders were collected in the same area and taken to the laboratory. These oviposited readily in captivity, thereby enabling us to secure an abundance of accurately-timed material.

Technique

Immediately after oviposition the egg sac was removed and an incision made in the wall of the cocoon to permit the removal of eggs. The cocoon was then stored in a small stoppered vial at room temperature. The work was carried out in the summer in a laboratory with a northern exposure—the temperature in the room rarely varying more than two degrees from the normal of 70° F. At regular intervals 6 to 10 eggs were removed and fixed. The following fixatives were used: 75% ethyl alcohol, Bouin's, Zenker's, and Susa's. After fixation the chorion was removed and drawings were made of the external features. In unstained material the embryonic tissues appear white by reflected light, while the yolk appears yellowish. Staining with paracarmine for a few minutes made the embryo stand out more clearly. The material was embedded in Tissuemat, sectioned at 6 micra, and stained by means of Delafield's Haematoxylin, with eosin as the counterstain.

Drawings of sections were made by projecting the image onto the drawing paper by means of a Bausch and Lomb bioscope. Most drawings were made on the same scale of magnification.

Oviposition

It is characteristic of the Aranea that at oviposition the egg mass is encased by a network of silken threads known as the cocoon. Considerable variations in cocoons and cocooning have been observed, but in general the female spins first a base to receive the eggs and then she forms a covering around the base and the egg mass (35). Cocooning generally takes place at night or in the early hours of the morning. Herms *et al.* (15) describe the process in the black widow.

The function of the cocoon is no doubt to protect the eggs and newly emerged spiderlings from desiccation and from enemies. Indeed, it is the protective cover of the cocoon and the great accumulation of stored yolk in the embryo that permit the latter to emerge from the chorionic envelope in an incompletely developed state. Moreover, part of the postembryonic development is completed within the cocoon, since the first instar lacks certain body structures and at best is capable of very limited movement. In some species even the second instar is incapable of movement. Holm (16) believes that originally all postembryonic stages of spiders were completely developed stadia, but that the "protective milieu" (cocoon) permitted successive reduction of body structures.

The number of cocoons produced in one season varies, but in the black widow the number appears to be around four, although Illingworth (23) records 15 cocoons produced by one female in captivity. The number of eggs per cocoon varies between 100 and 900 according to Kaston (27), while Blair (3) gives the limits as 100 to 600. In general the number is large in early cocoons and becomes smaller with succeeding cocoons.

The Egg

The egg is spherical and approximately 0.8 mm. in diameter. The chorion is thin, elastic, and smooth.

A careful examination of the egg of *L. mactans* has failed to disclose a micropyle. According to Savory (44) the spider egg lacks a micropyle and the chorion offers no resistance to the passage of the spermatozoon, which may penetrate the egg at any point. This fact would account for the frequent reference in the literature to polyspermy in spiders. In *Opilio parietinus* Deg., however, Holm (17) describes a micropyle.

The vitelline membrane (Fig. 4) is thin and transparent. During the first few hours after oviposition it adheres closely to the underlying periplasm and the overlying chorion (Fig. 1). Hence removal of the chorion in early stages is difficult. Soon, however, fluid separates the membrane from the chorion. In fixed and preserved material the removal of the chorion in eggs several hours old presents no difficulty. Beneath the vitelline membrane is the thin layer of periplasm overlying the yolk (Fig. 2). In *L. mactans* it is distributed fairly evenly over the egg (Fig. 4). This differs markedly from a condition found in *L. hasselti* as described by Sivickis and Filoteo (47). In the latter species the periplasm is concentrated largely at the animal pole. Since the periplasm is under pressure by the vitelline membrane from above and the yolk spherules from below, it is divided into fields. At various points the cortical cytoplasm penetrates the yolk in the form of fine strands that permit it to communicate with the nuclear cytoplasm. The presence of such reticular network has been generally assumed by workers of spider embryology. In sectioned and stained material it is difficult to detect. However, its presence in live material has been definitely established by Holm (18), who believes that its function is not only to permit the two plasma masses to communicate with each other, but that it also plays a role in the outward migration of the cleavage nuclei. Most of the interior of the egg consists of yolk in the form of globules of varying size. At the time of oviposition there is no definite pattern in the arrangement of the globules (Fig. 1). However, a little later the globules near the periphery assume a radiating linear arrangement, while the globules near the center form a solid mass (Fig. 5). A similar situation for another theridiid spider has been described by Montgomery (37). Later, during early cleavage, yolk columns become grouped to form pyramids (Fig. 7). Still later, when cleavage is complete and the cleavage cells have reached the periphery, the yolk becomes rearranged again, now to form yolk spheres (Fig. 9). Each yolk sphere will eventually become associated with a vitellocyte.

At the time of oviposition the nucleus is located somewhat off center of the egg. This, however, does not appear to be an indication that polarity is established at this early date. As in spiders generally, a gelatine-like material surrounds the eggs at the time of oviposition. In the writer's opinion this material causes the eggs to adhere to each other and to the silken cup. Later, when the egg mass is enclosed by a silken cocoon, the material dries, permitting

the eggs to roll around freely. According to Savory (44) the oviposition fluid is slightly alkaline, sirupy in consistency, with numerous small round particles in suspension. Warren (52) refers to it as a lubricating fluid, and his description agrees with that given by Savory. Upon evaporation of the fluid the granules adhere to the chorion, imparting to the eggs a hoary appearance. According to Holm (16) two types of granules are involved. One type is present at the time of oviposition and is formed shortly after the chorion is laid down. A second type is present in the oviposition fluid and is deposited on the surface of the chorion after the fluid has evaporated.

There has been much speculation regarding the function of the oviposition fluid. As stated already, the writer believes that in *L. mactans* it serves to hold the eggs together and causes them to adhere to the inner lining of the inverted silken cup into which the eggs are laid. After the cocoon is spun, the liquid evaporates.

Maturation and Fertilization

In spite of the examination of a large number of eggs shortly after oviposition, the writer has little data on maturation. In some eggs, a few minutes after egg laying, a nucleus is seen somewhat off center (Fig. 1). This presumably is the female nucleus prior to maturation. In ova about 10 minutes old, the nucleus approaches the periphery. At this time the nuclear cytoplasm has greatly increased and its contact with the periplasm is clearly evident (Fig. 2). In one ovum at 15 minutes the nucleus is near the periphery and is in the late prophase stage of division (Fig. 3). However, it has been impossible to get further information on maturation. In *Theridium* Montgomery (36) found that the first maturation division occurs during the first 15 minutes after oviposition and the second between 25 minutes and 1½ hours.

The next stage in my material is an egg at 4 hours with the female and male pronuclei close together (Fig. 4). The nuclei have increased in size until, at this stage, they have a diameter of nearly 15 μ. It is impossible to say with certainty which one of the two nuclei is the male pronucleus, and which is the female pronucleus. Following the example of Montgomery (36) we assume the larger of the two nuclei to be the male pronucleus. Since the nuclei are in close proximity to each other, fertilization must be near at hand. In *L. mactans* then fertilization occurs between 3 and 4 hours.

Polyspermy is a common occurrence in spiders. In *L. mactans* the writer has never found more than one sperm in the cytoplasm of the egg, but he has often found large numbers between the chorion and the vitelline membrane. Here they remain long after fertilization, even until the development of the embryo is well advanced. The location of these sperm cells suggests that in *L. mactans* the vitelline membrane exercises control over the entrance of the male gamete and that only one is permitted to enter the body of the egg and effect fertilization. This agrees with Montgomery (37) when he notes that the 'extra' sperms "always remain at the periphery".

Cleavage

In the zygote (Fig. 5, *zyg*) the nucleus is located near the center of the egg, and is surrounded by a large and fairly compact mass of yolk. Toward the periphery the yolk spherules are arranged in a radial manner to form columns (*ycol*). Such arrangement has been noted in other spiders and it has been assumed (Holm (16)) that it is due to a change in position of the reticular strands following cleavage. However, in the black widow spider this radial arrangement precedes cleavage, for it is already clearly evident in the zygote.

Cleavage commences at about 9 hours. At first a high degree of synchrony is observed in division. Thus at the second division, Fig. 6, all cells are in the metaphase. As cleavage progresses, the synchrony becomes less pronounced. In an eight-celled stage, three cells were found to be in prophase, one cell in metaphase, three cells in telophase, and one cell had just completed division. After the fifth cleavage, division becomes irregular. It should be pointed out, however, that some degree of synchrony in cell division is found even in advanced stages. Thus it is difficult to locate mitotic figures in advanced material, but when mitoses are encountered, their number is generally considerable.

Cleavage results not only in an increase in cells, but also in an outward migration of cells (Fig. 8). This movement is at first at a uniform rate, but as division becomes irregular, the outward migration also becomes irregular. Thus in a 32-cell stage, some nuclei have reached the periphery and have entered the periplasm, while other nuclei have traversed only half the distance from the center to the periphery. At 25 hours, after the seventh division, all nuclei are at the surface forming a blastema (Fig. 9, *blt*). Five hours later, cell walls appear. In *L. mactans*, as in spiders generally, the blastoderm (Fig. 10, *bld*) forms a complete envelope for the egg. Hence the observations on *L. hasselti* by Sivickis and Filoteo are quite at variance with what we know of blastoderm formation in spiders. Since in Theridiidae the blastoderm 'contracts' early to form the germ disk, it is possible that these authors have mistaken the germ disk for blastoderm.

In *Latrodectus* all cleavage cells migrate to the periphery and the vitellocytes are derived from cells of the outer and the inner layer at a later stage. This, too, is characteristic of spiders in general, but differs from the condition found in *A. labyrinthica* as described by Holm (16). In the latter species some cleavage nuclei fail to migrate to the periphery and become associated with yolk spheres as vitellocytes.

Coincident with cleavage, a further rearrangement in the yolk takes place. Several neighboring columns fuse to form a cone or pyramid (Fig. 7, *ypr*). The number of pyramids, however, does not correspond to the number of cleavage nuclei. Moreover, since the yolk of the pyramid lies *outside* a cleavage cell, it is incorrect to speak of 'total' cleavage. As pointed out by Holm (16),

> "Von einer totalen Spaltung des Spinneneies kann deshalb nicht die Rede sein."

When the yolk is arranged in pyramids, a small cavity may appear in the center. This is transitory, and when cleavage is completed, it disappears. The yolk spherules now undergo their final rearrangement whereby several spherules fuse to form a yolk sphere (Fig. 10).

Development of the Germ Disk

In *L. mactans* the blastoderm is of short duration. The cells, which are greatly flattened at first (Figs. 10 and 11), soon become columnar in what will be the central region of the germ disk (Figs. 10 and 12). The appearance of the germ disk can be ascribed to a contraction of the blastoderm, leaving part of the yolk uncovered. In *Latrodectus* the entire blastoderm takes part in the formation of the germ disk. Hence the extraembryonic area is poorly developed in this species, consisting largely of isolated cells. This also accounts for the well-known fact that in the Theridiidae the germ disk is an area with clearly defined margins (Figs. 13 and 15, *gd*).

Coincident with the appearance of the germ disk, an invagination is found in the central region, which has been referred to as the blastopore by Montgomery (37) and Primitivgrude by Holm (18). In *L. mactans* this invagination is not found in whole material, but it is evident in sections (Fig. 14, *blp*). Holm (18) finds it in live eggs, but states that fixation obliterates it. Immediately beneath the blastopore we find a group of large cells, which, judging from their position, have arisen through an inward migration of blastoderm cells (Fig. 14). The cells are larger than the overlying cells, probably because of the more intimate contact with the yolk and because of more active absorption of yolk. These cells are called the primary entoderm and their formation should mark the beginning of gastrulation. This event occurs at about 50 hours when the germ disk is in the form of a hemisphere (Figs. 15 and 16, *gd*).

The increase in the number of cells below the germ disk continues and 5 to 10 hours later forms an accumulation of cells referred to as the primitive cumulus (Figs. 17 and 18, *cp*). The cumulus is located at the future caudal end of the germ band. Since we do not know what part of the cumulus is future entoderm, and what part is future mesoderm, we shall refer to the cells as mesoentoblast cells (Fig. 19, *mesbl*). The cells of the outer layer then become ectoblast cells (Fig. 20, *ectbl*). When the embryo is 90 hours old, the cumulus separates from the remainder of the mesoentoblast and shifts caudad and dorsad (Fig. 19, *cp*).

The germ disk still occupies nearly a hemisphere of the egg at 100 hours. In whole mounts under reflected light (Fig. 21), the central region is lighter in color than the edge. The lighter area can again be seen to consist of two regions: a small one, representing the location of the primitive cumulus (*cp*), and a large one (*mesbl*), indicating the extent of distribution of the inner layer, the mesoentoblast. A longitudinal section of this stage is represented in Fig. 22.

During the next 10 to 15 hours, cellular activity is high and numerous mitotic figures are found in both the outer and the inner layer (Fig. 20). The shape and position of the cells and the direction of the mitotic figures indicate that the mesoentoblast increases in extent through cell division and through cell immigration from the outer layer. This differs from the condition found in *Theridium*. Montgomery (37) fails "to find indications that these mesoentoblasts develop *in situ* from overlying ectoblasts. All the mitotic spindles of the ectoblast seem to be placed horizontally and none vertically at this stage."

At this time, i.e. at approximately 110 hours, a few cells separate from the inner layer and migrate into the yolk. These are the yolk cells or vitellocytes. Before they break from the inner layer, they grow in size until the nucleus has increased as much as eightfold. The number of vitellocytes entering the yolk is small at first, but when the embryo is 160 hours old one can see the emigration of vitellocytes in large numbers.

In *L. mactans*, then, vitellocytes arise from mesoentoblast and ectoblast cells. Figs. 24, 25, 27, 28, 30, and 30A show the origin of yolk cells from mesoentoblast cells, while Figs. 26 and 29 show the appearance of yolk cells at the margin of the germ disk. The above figures represent sections of a 160-hour embryo as shown in Fig. 23. At 165 hours the greater mass of the yolk is still devoid of yolk cells, but many enlarged cells are found leaving the inner layer and the margin of the ectoblast. This mode of origin contrasts with that found in *Theridium* where the vitellocytes wander in from a single point of origin (Montgomery (37)), but agrees entirely with the condition in *Agelena* as described by Holm (18),

> "Von der Primitivplatte und auch von der Keimscheibe ausserhalb dieser werden von Anfang an recht zahlreiche Vitellozyten abgeschieden, die in die Dottermasse einwandern."

At 200 hours vitellocytes are found widely distributed throughout the yolk.

Gastrulation

In studies of arthropod embryology the subject of gastrulation has received much attention and aroused much controversy. The problem is twofold: First, do the vitellocytes eventually become part of the embryo or is their role merely that of preparing the yolk for assimilation? Second, what part of the inner layer is destined to become entoderm and what part mesoderm? A lengthy review of the subject is given by Holm (16). The problem is still an open one, awaiting further studies.

In *L. mactans* the blastoderm is completed at 25 hours. Two hours later it has contracted to form the germ disk, leaving much of the yolk uncovered. As in other Theriidae the germ disk is a well-defined area. Whereas in *Agelena* the early germ disk appears to be entirely presumptive mesoentoderm, in *L. mactans* it represents all three future body layers. It is, however, possible that there is no disagreement here. In *Latrodectus* the shift from the animal pole to the vegetative pole is very rapid and the germ disk is well differentiated

before an inward migration of mesoentoderm takes place. It is conceivable that in the early blastoderm the cells near the future blastopore are presumptive mesoentoderm, for this region contributes the cells that migrate inward to form the inner layer.

Subsequent events in the process of gastrulation are similar in the two species. The primitive cumulus separates from the inner layer and migrates to the edge of the germ disk, where it disappears, while the remainder of the inner layer (Analpol of Holm) causes the germ disk to become two-layered. It should, however, be stressed that in *Latrodectus* the inner layer grows in extent not only through cell proliferation, but also through cell immigration from the overlying outer layer. It is also of interest that in *Latrodectus* the vitellocytes, by some authors considered to be entodermal cells, arise not only from the inner layer but also directly from the outer layer at the edges of the germ disk. Not until 200 hours have elapsed does the production of vitellocytes cease. This period coincides with the appearance of segmentation.

It is difficult to trace the origin of each of the three body layers. In *Latrodectus* the first cells to migrate below the surface during gastrulation have been referred to as mesoentoderm cells, following the procedure adopted by many other workers, notably Holm. The outer layer, however, at this time, can hardly be designated as ectoderm, since its cells continue for some time to produce further mesoentoderm cells and also vitellocytes. The term ectoblast would seem to be more appropriate. Finally, the term "entoderm" has significance only if we can establish the origin of the mesenteron.

Kautzsch (29) in discussing the embryonic origin of the middle and distal regions of the mesenteron in *Agelena* finds that the cloaca and the yolk sacs are formed from outward migrating yolk cells, while the intestine arises as an outgrowth of the cloaca. My observations are in complete agreement with these findings (see below). Other workers, such as Schimkewitsch (46), derive the mesenteron from entoderm primordia. Korschelt and Heider (30) are inclined to accept this interpretation. Unfortunately this offers no solution to the problem, for nothing is learned regarding the origin of these primordia.

The germ-layer theory, so useful in vertebrate embryology, has been so difficult to apply in arthropod embryology that some authors are inclined to question its utility, even validity, in arthropods. Thus Kautzsch (28) states,

> "In vergleichend entwicklungsgeschichtlicher Hinsicht aber besitzt der Begriff des Entoderms bei den Arachnoideen, wie wir sahen, kaum noch eine Bedeutung."

Since in arachnid embryology it has been shown, repeatedly in the past and again by the writer, that the vitellocytes give rise to the mesenteron, the question of the status of the yolk cells arises. Are they to be considered primary entoderm, or are they nongerm-layer derivatives? If they are not to be considered entoderm, then the term "gastrulation" has little meaning in arthropod embryology and Kautzsch (28) is justified in stating that "Wir haben kein Recht, hier von einer Gastrulation zu reden".

In my opinion, the yolk cells in spiders must be assigned the status of entoderm and the formation of the mesenteron is a belated phenomenon merely because of the presence of an unusually large amount of yolk. An early differentiation of a mid-gut in spiders would be a hindrance, rather than an asset. This view is quite in harmony with those of experimental embryologists. To quote Richards (43),

> "Whatever cells in each particular case happen to be appropriately located at the time when the entodermal strands are due to be formed, those cells will be "determined" to form the mid-gut anlage and subsequently differentiate into the structure typical of mid-gut epithelium."

That vitellocytes give rise to the mesenteron in other arthropods, for example insects, is evident. Johannsen and Butt (24) point out that in some Orthoptera, Trichoptera, Odonata, and Thysanura the mid-gut epithelium is reported to be derived from yolk cells. If a careful re-examination of the origin of the mesenteron in a long series of arthropods were to give further evidence in favor of our view, then the term "gastrulation" could be applied appropriately in the arthropods, using the term in accordance with its original meaning.

Development of the Segmented Germ Band

When gastrulation is concluded, the germ disk still occupies nearly one hemisphere of the egg. At 150 hours the disk becomes heart-shaped (Fig. 31). During the next 10 hours it lengthens and changes into a germ band (Figs. 32 and 33). The outer layer is made up of closely packed cells. The inner layer, only one cell thick, is not continuous. The cells are similar in shape to the former, but somewhat larger (Fig. 34). At this time many vitellocytes can be seen in the interior of the egg.

At 190 hours further growth in length has occurred and the first indication of segmentation is evident (Fig. 34). The first somites are found in the prospective thoracic region, namely the points where the pedipalps and the first three pairs of walking appendages will develop. Soon the segment of the fourth pair of appendages becomes distinct. Then follow the cheliceral and the first abdominal segments and in rapid succession the rostral and the remainder of the abdominal segments until the definitive number of eight is reached.

When segmentation becomes evident, the germ band is already very long, reaching two-thirds around the circumference of the egg (Fig. 34). The inner layer is still only one cell thick and there is no evidence of its separation into somatic and splanchnic layers. Although many vitellocytes have become intimately associated with yolk spheres, some are still found as large free cells between the yolk spheres.

During the next 10 hours development proceeds rapidly. As segmentation of the outer layer becomes more pronounced, the inner layer divides into segments, a pair for each somite (Fig. 35). It is my impression that the

appearance of the coelomic sacs and the division of the mesoderm into splanchnic and somatic layers is the result of a rolling-in process (Figs. 35C and 36). Such origin of coelomic sacs is in agreement with events occurring in *Theridium* as described by Montgomery (37). However, Korschelt and Heider (30) in their outline of arachnid embryology describe the appearance of coelomic sacs as a result of delamination. Development is most rapid in the thoracic region. Thus, at 235 hours the coelomic sacs are completed in the segments of the pedipalps and the first, second, and third pairs of legs. This is diagrammatically represented in Fig. 35B.

Almost coincident with the appearance of the thoracic somites, outgrowths of the future appendages appear. The sequence of events can be followed by observing changes in the external form of the embryo. At 230 hours (Fig. 39) the regions most advanced are found in the areas of the thorax and anterior abdominal segments. The cephalic region is least advanced. Hence the leg appendages are more prominent than the appendages of the pedipalpal and cheliceral segments. The rostral appendages (*lm*), which later fuse to form the labrum, are made out with difficulty (Fig. 40). The growth of appendage rudiments is followed by growth of the mesodermal masses into the rudiments. In the thoracic region, at this time, the mesoderm comes to be restricted largely to the appendages. A similar situation exists in abdominal segments two to five.

All the appendage rudiments are at first directed caudad (Fig. 37). However, as development proceeds, they rotate through 90° toward the median line (Fig. 38). At 250 hours (Fig. 42) the rotation is complete. This state is further characterized by the presence of two faint rostral (*lm*) appendages and by further division of the abdomen. The latter now has seven segments. Fig. 42 shows a ventral view of the cephalothorax, Fig. 43 the lateral aspect, Fig. 44 a cross section, Fig. 45 a parasagittal section, and Fig. 40 the embryo as it would appear if removed from the egg and straightened out. The embryo is very long, almost encircling the egg. Coelomic sacs are well developed in every segment, although in the appendage-bearing segments the mesoderm is almost entirely restricted to the appendage (Fig. 45). No stomodaeal invagination is found in this stage. Of special interest is the presence of a large number of cells underneath the chorion (Fig. 50, *spr*). Since these cells are separated from the embryo by the vitelline membrane, they must be sperm cells. The presence of supernumerary sperms has been observed in many stages. Of further interest is the presence of very large cells just inside the lateral embryonic body wall (Fig. 50, *yc*). The position of these cells suggests that they arise from proliferating ectoblast cells along the margin. A similar situation is observed by Montgomery (37), who looks upon the cells as vitellocytes.

During the next 20 hours further changes involve the appearance of abdominal appendages on segments two to five and the separation of the seventh abdominal segment from the caudal lobe (Fig. 41). The rostral appendages have moved cephalad and fused; a small depression behind the

rostrum signifies the primordium of the stomodaeum. It is significant to record that a number of workers in arachnid embryology have described the origin of the rostrum (= labrum) from two appendage-like outgrowths. Croneberg (10) derives the rostrum in spiders from a pair of primordia. Montgomery (37) states that in *Theridium* at the anterior border of the stomodaeum there "is a pair of small, basally contiguous prominences, the rostral appendages; later these will fuse to form the rostrum . . .". Montgomery figures coelomic sacs appertaining to the rostral appendages and he comes to the conclusion that these prominences can be properly considered prestomial appendages and that they may be rightly adjudged cephalic appendages. Other authors who have demonstrated the origin of the rostrum from a pair of cephalic prominences are Schimkewitsch (45) and Lambert (31). The figure given by Lambert (Fig. 21, Lambert) shows quite clearly that the rostrum is derived from two prominences. Lambert also states that "in sections these elevations are seen to be lined with mesoderm, and enclose a portion of the coelomic cavity, a fact which indicates their appendage-like character." Wallstabe (51), in his figures, shows the presence of a precheliceral coelom. In the insects, too, numerous cases are known where the labrum (= rostrum) arises through the fusion of two distinctly separated prominences. The subject has been reviewed at great length by Butt (7).

It is generally agreed that before an appendage-like characteristic can be ascribed to the rostral prominences, it must be shown that they possess separate coelomic sacs. As pointed out above, several authors have laid claim to finding such coelomic cavities. Korschelt and Heider (30), however, state that the cephalocoelomic cavity may be merely an extension of the cavity of the cheliceral segment. With this in mind the writer made a careful study of his material and has come to the conclusion that in *L. mactans* there is definitely present a cephalic coelomic cavity (Figs. 46, 47, and 48). The presence of such a distinct cavity can be ascertained in longitudinal and cross sections. Stages just prior to reversion and those at the beginning of reversion show underneath the thickened cephalic ectoderm a mesoderm enclosing a spacious coelomic cavity, distinctly and widely separated from the cheliceral segment. This finding is of interest in view of the commonly accepted origin of the arthropod labrum from a non-appendage-bearing cephalic region.

The appendages continue to increase in length and at 290 hours (Figs. 52 and 53) they overlap. At the base of the pedipalp the first indication of a "maxilla" is found in the form of a small basal lobe. The chelicerae have moved cephalad and mesad, but are still postoral in position. The head lobes are large and prominent. The embryo has now reached its maximum length and almost completely encircles the egg (Figs. 49 and 53). The developing nervous system shows as a series of paired thickenings near the median line and as the large cephalic lobes.

At this time also, large cells appear immediately below the 'extraembryonic' layer (Fig. 51, *bl*). These are the first blood cells. Their position suggests

that they derive their origin from proliferating extraembryonic cells. In other words, they are ectodermal in origin. Although extraembryonic cells have not been observed in the process of division, the description of the origin of blood cells in *Theridium* as given by Montgomery (37) supports my view. Moreover, when the blood cells first appear, they are far removed from what are definitely known to be the mesodermal cells (Fig. 51, *mes*). Only during the process of reversion (later to be described) do the blood cells become closely associated with mesoderm. According to Korschelt and Heider (30) most workers derive the blood cells from yolk cells, but I have not noticed any outward migration of yolk cells at this time. Moreover, if extraembryonic cells can give rise to vitellocytes, which later are supposed to change to blood cells, it would seem reasonable to assume that extraembryonic cells could develop into blood cells directly.

It is obvious that this view contrasts with the commonly accepted theory of the mesodermal origin of the blood. It should, however, be pointed out that in later stages 'haemapoiesis' is a function of the heart muscle, the latter being, of course, a mesodermal derivative. Because of their large size and granular cytoplasm, the blood cells are readily recognized and the further course in their developmental history is readily traced (Fig. 74, *blc*).

Reversion

In spiders the embryo during much of its development is long and narrow, almost encircling the yolk. Although the cephalic and caudal ends nearly meet dorsally, laterally much yolk is left uncovered by embryonic tissue. The function of the process of reversion then is to move the lateral edges of the body wall dorsad to enclose the yolk. Reversion performs a second function in that it brings about a change in the curvature of the spider from the embryonic to that of the adult.

Reversion has been studied by numerous authors. In a lengthy review of the subject Holm (16) divides the spiders on the basis of reversion into two types. In the first type the postabdomen is directed ventrad and forward. The families Dysderidae and Pholcidae belong here. In the second type the postabdomen is directed dorsad and caudad. Most families of spiders including the Theridiidae fall into this category.

In *L. mactans* reversion sets in at approximately 300 hours (Fig. 54). The ectoderm along the mid-ventral line of the abdomen becomes attenuated to form the ventral sulcus (*sulv*). The latter will gradually extend forward as far as the stomodaeum and backward as far as the telson. In the region of the ventral sulcus the ectoderm becomes sufficiently thin to show the underlying yolk spheres. As the sulcus widens, the lateral plates move dorsad until a fusion of the outer edges, along the mid-dorsal line, is achieved.

In the 330-hour stage (Fig. 55) the ventral sulcus extends anteriorly to the labrum and undergoes further sidewise extension in the abdominal region (Fig. 56). The labrum (*lm*) is now a large single structure overhanging the stomodaeum. The latter (*stom*) now forms a deep ectodermal infolding which

distally pushes into a mass of mesodermal tissue (Fig. 58). In cross sections of the stomodaeum the mesodermal cover is seen only if the section is taken near the end (Fig. 60A). The chelicerae are still postoral in position, but have become more widely separated. The pedipalp and leg extremities for the first time show an indication of segmentation. The appendages of abdominal segment two (Fig. 60, *blg*) soon show infoldings of the ectodermal layer. These folds foreshadow the appearance of the book lungs.

The blood cells increase in number and form a layer between the ectoderm and the yolk (Fig. 59, *bl*). During reversion the mesoderm grows rapidly dorsad from the ventral region of the embryo. As a result the blood cells soon become separated from the ectoderm by somatic and splanchnic layers of mesoderm (Fig. 57). With further advance in reversion the blood cells shift toward the dorsal and posterior end of the abdomen, the future site of the heart (Figs. 58, *ht*).

The widening of the ventral sulcus causes a temporary separation of the nerve ganglia (*gleg*). This laterad shift is especially pronounced in the regions of the thoracic appendages (Fig. 59) and the anterior abdominal segments (Fig. 56). The separation in these regions may extend over two-thirds of the circumference of the egg.

The progressive widening of the ventral sulcus and the covering of the extraembryonic area during the next 30 hours is illustrated in Figs. 61, 64, 65, and 66. The fusion of the lateral plates will first occur in the region of abdominal segment eight, then seven, six, five, and four follow (Figs. 62 and 63). Somite eight at the time of fusion encloses the caudal lobe (*tel*). The latter will form the anal tubercle (Fig. 63). The closure dorsally is not complete until 360 hours (Fig. 66). Coincident with the dorsad extension of the lateral plates of the embryo, a shift in position of the chelicerae takes place. Until now located postorally and laterally to the labrum (Fig. 63, *lm*), they move first forward and then mesad in front of the labrum (Fig. 66). This accounts for the preoral position of the chelicerae in the young and adult spider.

With the disappearance of the dorsal extraembryonic area, a growth in the reverse direction sets in. The dorsal body wall of the abdominal region grows caudad and then ventrad (Fig. 67). This causes a return of the abdominal 'sternites' to the ventral surface and a return of the abdominal ganglia to near the mid-ventral line. The abdominal appendages of segments two and three have regressed and are now no longer visible in surface view. The appendage of segment two, however, will continue to undergo extensive internal changes. The infoldings, which start at the beginning of reversion (Fig. 60, *blg*), become deeper and more numerous to produce the lamellae of the book lung. An invagination at the base of the appendage of segment three forms the tracheal tube. Since in some spiders the respiratory organs on segments two and three are lungs (Tetrapneumones), in others tracheae (Caponiidae), while in the majority the anterior ones are lungs and the posterior tracheae, it follows that lungs and tracheae are serially homologous structures, the lung being the ancestral organ (Snodgrass (48)). The

appendages of segments four and five represent the primordia of the anterior and posterior spinnerets.

At 370 hours the shift of tissues toward the mid-ventral line is completed, thereby bringing reversion to its conclusion (Fig. 68). Duplication of embryonic tissue, as in siamese twin embryos, is thought to interfere with reversion and thereby to terminate development (Rempel (42)).

Development Following Reversion

When reversion is complete at 370 hours, the two main body regions of the future spider, namely prosoma (or cephalothorax) and opisthosoma (or abdomen), are already clearly evident (Figs. 68 and 69). As a result of the lengthening of the dorsal body wall, the ectodermal and mesodermal cells here have become greatly flattened (Fig. 70) and in some areas the body wall is so thin that the underlying yolk spheres can be seen in whole material (Figs. 68 and 69). Coincident with the lengthening of the dorsal body wall, the ventral wall contracts, causing the ectodermal cells to become columnar (Fig. 70). Moreover, the concentration of tissues on the ventral side facilitates the appearance by invagination of the organs associated with respiration, reproduction, and spinning of silk.

The nervous system has undergone further concentration and development. Above the stomodaeum are found the protocerebrum (*pcb*) and the cheliceral ganglia; below the stomodaeum are the pedipalpal and leg ganglia (*gleg*). The abdominal ganglia are still located near the seat of origin (Fig. 70, *nc*) and their withdrawal from the abdomen does not become evident until the embryo is 380 hours old. The stomodaeum has become greatly extended in length and a thin layer of mesoderm covers its distal enlargement. Anteriorly the mesodermal cover increases in thickness (Fig. 71, *mes*).

The shift of the mesoderm from the lateral edges dorsad and mesad, and from the caudal region forward, produces a large blood sinus with long lateral arms. The latter get progressively shorter until the system assumes its definitive form of a vessel, the heart. When the mesodermal layers from opposite sides approach each other along the mid-dorsal line, the somatic layers fuse first, followed shortly by a fusion of the splanchnic layers. This mode of formation of the heart permits the inclusion of the blood cells in the vessel (Fig. 72). During the next 10 hours the heart muscle develops and the pericardial sinus becomes differentiated (Fig. 73, *pers*).

Almost coincident with the formation of the heart, mesoderm grows forward from the region that will form the dorsal boundary between prosoma and abdomen toward the upper edge of the cup-like inner end of the stomodaeal invagination (Fig. 70). As a result a considerable amount of yolk is enclosed in the embryonic prosoma. This is obviously an adaptation to facilitate the absorption of yolk by cells in the region of very active cell differentiation. At the same time the splanchnic layer of the mesoderm forms folds in the abdomen that penetrate the yolk deeply in a number of places. This is the first indication of the appearance of septa, which later divide the yolk into

yolk sacs (Magenblindsäcke or Leber of German authors). It is significant to point out that these septa originally are entirely of mesodermal origin and that the appearance of the entodermal lining is greatly delayed in spiders. It has been generally assumed (Kautzsch (28)) that these septa arise at the boundary lines between the embryonic abdominal segments. This is the case in *L. mactans* (Fig. 76). Since the splanchnic and somatic layers are closely applied to each other, the mesoderm at first glance appears to be a single layer. However, at the base of the septum the two layers are made out with ease (Fig. 70B, *splm* and *somm*). At this time also, invaginations in the chelicerae (Fig. 70A, *pgl*) disclose the origin of the poison glands, while infoldings at the posterior margin of the second abdominal appendage indicate the formation of the book lungs. A thick cuticular structure at the end of the pedipalp (Fig. 75) represents the egg tooth (*egt*).

In the posterior region the cloaca or stercoral pocket is now formed. According to Comstock (9), the latter structure is ectodermal in origin and constitutes part of the hind-gut. This, however, is incorrect as an examination of the histology of the structure in the adult reveals. The wall of the cloaca is a syncytium with the inner margin heavily ciliated. It is not lined by a cuticular intima. Snodgrass (48) treats the cloaca correctly as part of the mesenteron. The most important contribution toward the ontogeny of the cloaca (= Rektalblase) is that of Kautzsch (29) who, in an extensive study of the embryology of the cloaca, the intestine, and the Malpighian tubules, shows clearly that the first part of the mid-gut to arise is the cloaca, from which later the intestine and the Malpighian tubules grow out as forward directed diverticula. We have been able to substantiate these findings in *L. mactans*. At approximately 380 hours the cloaca is found as a round hollow body dorsal to the posterior spinnerets. During the next 10 hours three outgrowths arise from the anterior surface, the median outgrowth forming the intestine (*int*) and the two lateral outgrowths developing into the Malpighian tubules (*malp*) (Fig. 76 K, L, M).

That the cloaca then is not an ectodermal derivative is evident from its histological structure and from the fact that it arises *prior* to the appearance of the proctodaeal invagination. Hence the belief of the early embryologists—Locy (32), Morin (38), and Pappenheim (40)—that the cloaca is ectodermal in origin is found to be incorrect. Although Schimkewitsch (46) and Montgomery (37) postulate the presence of a posterior mesenteron rudiment, evidence from embryology is lacking. Kautzsch (29) traces the development of the cloaca from yolk cells, and his descriptions and illustrations are most convincing. I am inclined to accept Kautzsch's explanation, but on circumstantial evidence. At *ca.* 350 hours the only cells that can be found in the abdomen besides ectodermal and mesodermal cells, which are already in various stages of differentiation, are the vitellocytes. That these cells produce the lining of the yolk sacs, which constitute part of the mid-gut, seems to be well established in the literature (Kautzsch (29)). In *L. mactans* the intersegmental mesodermal septa arise at approximately 370 hours. Almost immediately

large cells appear in the neighborhood of these septa. Since these cells are definitely not mesodermal cells, they can only be vitellocytes that have been stopped in their outward migration from the yolk by the septa. Moreover, these cells are at first especially numerous in the posterior region of the abdomen. I, therefore, believe that in spiders the origin of the mesenteron can be explained in the following manner: Outward migrating vitellocytes lay down the primordium of the cloaca from which, in turn, arise the intestine and the Malpighian tubules. A little later further vitellocytes migrate against the mesodermal septa (Fig. 76). These cells form the lining of the yolk sacs. Kautzsch (29) derives the lining of the yolk sacs from cells of the septa, but claims that a separation of entodermal and mesodermal elements is impossible. In my material such difficulty has not been encountered. The septa are made up of greatly flattened cells joined to each other by a thin layer of cytoplasm, while the cells that form the lining are large round cells with large round nuclei. The latter cells are found not only lying against the septa, but also some distance from the septa, amongst the yolk spheres. Since they are moving outward, they cannot be derived from the septa. (Fig. 76 A, B, C, H, I, J).

If there are difficulties in explaining the origin of the posterior and the middle primordia of the mesenteron, the origin of the anterior rudiment has been even more difficult to account for. Kautzsch (29), who deals with the origin of the mesenteron in great detail, fails to discuss it. Hamburger (13) derives it from an anterior entodermal rudiment. Unfortunately her account throws no light on the origin of this rudiment. My material indicates that the anterior region of the mesenteron also finds its origin in an accumulation of vitellocytes, but the appearance is much delayed (Fig. 79, *ames*). The sequence of events in the development of the digestive canal then is as follows: First to appear is the stomodaeal invagination; then to follow the cloaca, the intestine, and the yolk sacs. The anterior region of the mesenteron and the proctodaeum are last to develop. A direct communication between the three regions of the digestive canal will not be established until some time after hatching.

Hatching

In *L. mactans* the developmental period from oviposition to hatching extends over 390–400 hours, or nearly 17 days. This agrees well with Deevey's (12) observation that hatching occurs 15 to 20 days after the eggs are laid. This period, however, can be markedly shortened or lengthened by high or low temperature (Blair (3)). As the time of hatching approaches, the organism is not only covered by the chorion and the vitelline membrane, but also by the loose cuticula of the embryo. Hence the hatching process in *L. mactans* appears to be similar to that found in other Theridiidae as described by Holm (16). Fig. 77 represents an embryo at the time of hatching with the chorion and vitelline membrane removed. Fig. 79 is a parasagittal section of the embryo showing the chorion, the vitelline membrane, and the loose embryonic cuticula.

The nervous system of the late embryo is restricted to the prosoma (Fig. 86). The brain consists of the protocerebrum (*br*) and the cheliceral ganglia (1). It is well known that in spiders the chelicerae move from an original postoral position to a preoral one in late embryonic development. In consequence their ganglia shift to a preoral position to unite with the protocerebrum, but they retain their postoral commissure. Hanström (14) homologizes the cheliceral ganglia with the tritocerebrum of the Insecta and therefore assigns them to the first cephalic segment. But in insects the tritocerebrum innervates the labrum, while in the arachnids the labral nerves arise on the stomodaeal bridge. Would it not then be possible to homologize the tritocerebrum with the stomodaeal bridge and consider the cheliceral ganglia as part of the second cephalic segment? We would then postulate for the spiders a syncerebrum made up of a protocerebrum and a greatly reduced tritocerebrum (= stomodaeal bridge), to which have been added the cheliceral ganglia, while in the mites, through further cephalization, the pedipalpal ganglia would also be associated with the "brain". It should be stressed that this view is entirely at variance with commonly accepted theories of arthropod segmentation (see Hanström), but it does enable us to derive the arthropod labrum from *two* embryonic appendage-like structures and it further removes some difficulties encountered in attempting to homologize the cephalic and thoracic appendages of the insect with the prosomatic appendages of the arachnid. The problem of arthropod segmentation has aroused bitter controversy in the past and a satisfactory solution is not yet in sight. The answer will be given eventually by the experimental embryologist.

In spiders cephalization affects the abdominal nerve ganglia to such an extent that the entire nerve cord shifts cephalad into the prosoma and unites with the suboesophageal nerve mass. This process of successive cephalization is illustrated in Figs. 76, 76 A–G, 79, and 86. The withdrawal of the nerve cord from the abdomen removes from this region a system which frequently affords landmarks of segmentation. Since in spiders, at this time, the abdomen is globose and smooth, all semblance of outward segmentation has disappeared. However, in sagittal section, the position of the septa and the muscles (Fig. 86) indicates the segments clearly. Dorsally eight segments and the telson are present; ventrally only the sterna of segments two, three, four, and five are found. A recent description of segmentation of the spider abdomen is given by Kästner (25) and Millot (34).

The eyes of spiders are of two types. The anterior median eyes, also known as postbascillary eyes (Hauptaugen of German authors), have the retinal nuclei behind the light-sensitive rods (Snodgrass (48)). This position is assumed through a process of involution during embryonic development (Fig. 91). The other eyes (anterior lateral, posterior lateral, and posterior median) have the retinal nuclei in front of the light-sensitive rods. They are called erect, converted, or prebascillary eyes by American authors and Nebenaugen by German workers. They have been the subject of an intense study by Homann (19, 20, 21, 22). In *L. mactans*, at the time of hatching,

the anterior median eyes are at the stage of involution (Fig. 91, *ameye*), while the other eyes show the retinal layer sink below the surface. Their mode of development and their stage of development at the time of hatching agree with the findings of Homann.

The muscular system of the young spider is well developed, but a systematic study of it here is beyond the scope of this paper. The musculature of the adult was recently worked out by Whitehead (53).

The development of the endosternites parallels that of muscles since the arachnid endoskeleton results from the tendinous solidification of muscle tissue (Pocock (41)). In the prosoma the endosternite forms a large plate-like structure for muscle attachment (Fig. 92, *endst*). In the abdomen (Fig. 86) endosternites develop at the posterior limits of segments two, three, four, and five. A detailed description of the endoskeleton of the adult black widow spider is given by Whitehead (53).

The first part of the respiratory system consists of a pair of book lungs made up of a large number of "leaves" which arise as infoldings along the posterior border of the appendages of the second abdominal segment (Fig. 89). Since the lungs are not functional until after hatching, the leaves are closely applied against each other (Fig. 87). The nuclei of each leaf are generally so spaced that opposite nuclei are in line and are connected by pillars of cytoplasm. Blood cells are found in the lacunae. In the adult the leaves are kept apart by a series of vertical spines. They are not yet developed at the time of hatching. Comstock (9) points out that the term "book lung" is a misnomer since the leaves are not free at the edges. Hence "the lung has been more aptly compared to that form of a letter-file that consists of an envelope divided by many partitions into numerous compartments". This condition is easily observed in a horizontal section of the lung (Fig. 88). The second part of the respiratory system consists of a pair of tracheae that arise as invagination at the posterior border of segment three (Fig. 80, *tsp*).

The reproductive system, at the time of hatching, consists of the gonads located just dorsad of the ventral longitudinal abdominal muscles. They extend forward from the fifth segment to the middle of the second segment and then open to the outside through their respective ducts in the region of the epigastric furrow (Fig. 80). Kautzsch (29) has located germ-cell primordia in *A. labyrinthica* before the completion of reversion, in the vicinity of the coelomic sacs of segments 3 to 6. Kautzsch (29) and Strand (49) are able to distinguish between the sexes at this early stage. I have, however, looked in vain for the distinguishing characters cited by these authors.

At the time of hatching the fore-gut is divided into the definitive three regions: pharynx, oesophagus, and sucking stomach or proventricular pump. In the pharynx (Figs. 81*a* and 82) a thickening of the dorsal plate is evident, showing the position of the dorsal channel. Closely applied to the ventral wall is found the ventral retractor muscle of the pharynx. The oesophagus (Figs. 81*b* and 83) is a simple tube that connects the pharynx with the sucking

stomach. The latter in cross section is shaped like a T, with three sets of muscles for dilation (Figs. 81*c* and 84) and one for constriction.

The anterior part of the mesenteron, which is poorly developed (Figs. 81*d* and 85) consists of closely packed somewhat columnar cells which apparently have come from vitellocytes. Outwardly the anterior part of the mesenteron is covered by a coat of mesoderm and is suspended from the dorsal body wall by two layers of splanchnic mesoderm. The mid section of the mesenteron is made up of the large yolk sacs, at this stage lined by large, isolated yolk cells and covered by a thin layer of splanchnic mesoderm. The posterior end of the mesenteron consists of the short intestine and the cloaca. Its development can best be studied in a series of cross sections (Fig. 80, A to I). The proctodaeum is short. Communication between the three divisions of the alimentary canal is not yet established (Fig. 76N).

The excretory system consists of two Malpighian tubules, arising as outgrowths from the cloaca (Fig. 80F, *malp*). Hence they are entodermal derivatives. Histologically the cloaca resembles the Malpighian tubules and for that reason is thought to play a role in excretion. The writer has not been able to make out nephrocytes, the coxal glands, nor the guanin cells associated with the mesenteron yolk caecae.

The circulatory system is now complete, consisting of a dorsal heart (*ht*) enclosed in a pericardial sinus (*pers*) (Figs. 78 and 80) and an aorta extending forward and sending branches to the organs and appendages of the prosoma. Fig. 78 shows the heart and the branches to the appendages in a horizontal section, while Figs. 92 and 93 show the blood filled branches as seen in a cross section of the prosoma. The wall of the heart is made up of an outer layer of longitudinal muscle and an inner layer of circular muscle. An endothelial lining is absent. The circular muscle fibers, which appear as muscle pillars in a parasagittal section (Fig. 90), are the seat of haemapoiesis. An excellent description of the morphology and physiology of the arachnid heart muscle is given by Millot (33).

The spinning apparatus of spiders consists of spinnerets and silk glands. The spinnerets are six in number—an anterior pair, a posterior lateral pair, and a posterior median pair. The anterior spinnerets are derived from the embryonic appendages of abdominal segment 4, the posterior lateral pair from the appendages of segment 5. When the embryo is ready to hatch, only these are present (Fig. 80 A). The posterior median spinnerets arise by splitting-off from the posterior lateral spinnerets (Wallstabe (51)). During embryonic development the silk glands arise as a result of extensive cell proliferation of the ventral ectodermal layer of segments four and five. Each gland is a tubular structure with one layer of cells grouped around a central canal (*sgl*, Fig. 80, G, H, and I). Gland formation sets in at about 360 hours and at the time of hatching is still quite incomplete. In spiders the great development and differentiation of the spinning apparatus is a postembryonic phenomenon.

Summary and Discussion

This is a study of the embryonic development of *L. mactans*, the black widow spider, from the time the egg is laid until the embryo is ready to hatch. An effort is made to correlate the internal changes with changes in external body form.

The female spider produces several cocoons in a season, with the number of eggs in each cocoon varying between 100 and 900.

The egg is sphere-shaped and approximately 0.8 mm. in diameter. The chorion and vitelline membrane are thin. A micropyle is lacking. There is no indication of polarity.

Maturation sets in shortly after oviposition. Fertilization occurs at about 4 hours. Cleavage follows immediately. All cleavage cells migrate to the periphery, forming first a blastema and then, at *ca.* 25 hours, a blastoderm.

The blastoderm contracts to form a distinct germ disk, the shift of cells being from the animal toward the vegetative pole.

The primitive cumulus appearing at 57 hours marks the beginning of the inner layer. At 90 hours it shifts to the periphery of the disk and at 110 hours it has disappeared.

Vitellocytes, arising from inward migrating inner and outer layer cells, first appear at about 110 hours and continue to be formed until the 300-hour stage is reached. They are considered to constitute the entoderm.

The inner layer grows in extent through cell proliferation and through cell immigration from the overlying outer layer. It is mainly presumptive mesoderm.

At 170 hours the germ disk is transformed into a germ band. Segmentation sets in at 190 hours, with differentiation beginning in the prospective thoracic region and extending progressively forward and caudad. Outer segmentation is followed by division of the inner layer into somites. Coelomic sacs seem to be the result of a 'rolling-in' process, rather than the result of delamination.

The appendages of the prosoma appear at 230 hours, and the abdominal appendages at 270 hours. All appendage rudiments are at first directed caudad, but soon rotate through 90° toward the median line. The labrum is derived from a pair of cephalic rudiments which later fuse, anteriorly to the stomodaeum. Owing to the presence of coelomic sacs, the view is advanced that these rudiments represent true appendages. As a consequence a change in the commonly accepted theory of arthropod segmentation is suggested.

At 290 hours the embryo reaches its maximum length and almost completely encircles the egg. A few hours later reversion sets in as the result of an attenuation of the body wall along the mid-ventral line to form the ventral sulcus. Progressive widening of the sulcus shifts the lateral plates dorsad. A fusion of the outer edges of these along the mid-dorsal line occurs at 360 hours, thereby terminating the first phase of reversion.

Coincident with the widening of the ventral sulcus in the cephalic region, the chelicerae shift first laterad, then forward, and finally mesad, to assume a preoral position.

The second phase of reversion completed in 10 hours is characterized by a growth ventrad and mesad. This process facilitates the appearance by invagination of the organs associated with respiration, reproduction, and the spinning of silk.

The nervous system begins as a pair of ectodermal thickenings in the head lobes and a pair of thickenings in each of the six prosomatic and eight abdominal segments. During reversion the cheliceral ganglia assume a preoral position and fuse with the cephalic lobes to form the brain. Soon after reversion the abdominal ganglia shift into the prosoma to fuse with the ganglia of the legs and pedipalps to form the suboesophageal ganglion. The primordia of the eyes are laid down shortly before hatching.

The heart and surrounding pericardium are formed by a fusion of somatic and splanchnic layers of mesoderm along the mid-dorsal line of the embryo. The first blood cells are derived from extraembryonic (i.e., ectodermal) cells, while later haemapoiesis is a function of the heart muscle.

The stomodaeum arises as an ectodermal invagination at approximately 290 hours. From it will be derived the pharynx, oesophagus, and proventricular pump. At 370 hours the splanchnic layer of mesoderm forms folds in the intersegmental regions of the abdomen. These folds penetrate the yolk deeply and form the mesodermal cover of the mid-gut yolk sacs. Vitellocytes now migrate outward and line up against the septa. Some of the yolk cells form the stercoral pocket (= cloaca), while others form the lining of the anterior and middle divisions of the mid-gut. The cloaca forms three forward directed diverticula, the median one producing the intestine and the lateral ones developing into the Malpighian tubules. The proctodaeal invagination is last to appear. Communication between the three regions is not established until some time after hatching.

The respiratory system consists of a pair of book lungs made up of lamellae that arise as a result of infoldings along the posterior margin of the appendages of abdominal segment two. The second part of the respiratory system consists of a pair of tracheae that arise as invaginations at the posterior border of abdominal segment three.

The reproductive system, shortly before hatching, consists of a pair of gonads that extend from the fifth abdominal segment to the middle of the second and open to the outside in the epigastric furrow. A separation of the sexes is impossible.

The spinning apparatus consists of spinnerets and silk glands. The former are derived from the embryonic appendages of abdominal segments four and five, the latter result from extensive cell proliferation of the ventral ectodermal layer of these segments.

At 400 hours the embryo is ready to emerge.

This study has revealed that the events in the embryonic development in *L. mactans*, the black widow spider of the Americas, closely parallel the events described for other species of spiders. Since the embryology of only one other species of *Latrodectus* is known, a comparison of the two types is warranted,

especially since the embryology of *L. hasselti*, as described by Sivickis and Filoteo, departs in some respects from that of *L. mactans*.

In *L. hasselti* the periplasm is said to form a thicker layer in what the authors call the animal pole. This contrasts with what has been found in spiders generally, namely, that the periplasm is of uniform thickness all around, and does not disclose polarity.

In *L. mactans* and spiders generally, the cleavage nuclei in their outward migration move in all directions and reach the periphery simultaneously, while in *L. hasselti* the migration is said to be toward the animal pole only. In *L. mactans* the blastema, and later the blastoderm, form a complete cover of uniform thickness, while in *L. hasselti* the blastoderm is limited to the animal hemisphere. Since the blastoderm in theridiid spiders contracts early to form a distinct germ disk, it is possible that Sivickis and Filoteo have actually missed the stage of early blastoderm development. Moreover, it is difficult to believe that the blastoderm could form outside the limits of the periplasm.

A characteristic feature in spider embryology is the primitive cumulus. It is generally stated that the cumulus appears at what will later be the caudal end of the germ band. That this interpretation is correct has been shown by Holm. But in *L. hasselti* the cumulus is supposed to be located at the cephalic end. Further, it is known that the cumulus, although it plays an essential role as an organizing center in early development, is of short duration. But Sivickis and Filoteo conclude that in *L. hasselti* it "never disappears but remains as the anterior, physiologically dominant region . . .". This is obviously inaccurate. The authors are also wrong when they claim that the primitive cumulus "gives rise to the cephalic lobe and other parts of the future anterior region of the animal".

Sivickis and Filoteo consider the stercoral pocket (cloaca) as part of the hind-gut, when in reality it is part of the mid-gut. They are wrong when they claim that it "arises as the prolongation of the anterior end of the proctodaeum". The cloaca makes its appearance before the proctodaeum.

It is thus apparent that a re-examination of the developmental history of *Latrodectus* was greatly needed in order to bring it in line with the wealth of information extant on arachnid embryology.

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Explanation of Lettering on Figures

aap — abdominal appendage
ab — abdominal segment
aleye — anterior lateral eye
ames — anterior mesenteron

bl — blood
blc — blood cell
bld — blastoderm
blg — book lung
blp — blastopore
blt — blastema
br — brain

ca — caudal artery
cc — cleavage cell
ce — cephalic lobe
ch — chorion
chl — chelicera
clo — cloaca
coel — coelom
cp — primitive cumulus
cu — cuticula

dc — dorsal channel
dpl — dorsal plate

ect — ectoderm
ectbl — ectoblast
egt — egg tooth
endst — endosternite
ent — entoderm
epgn — epigynum
ext — extraembryonic area
eye — eye

fov — fovea

g — ganglion
gab — abdominal ganglion
gb — germ band
gchl — ganglion of chelicera
gd — germ disk
gdt — genital duct
gleg — ganglion of leg
go — gonad
gpdp — ganglion of pedipalp
gsub — suboesophageal ganglion

ht — heart
htmcl — heart muscle

inl — inner layer
int — intestine

leg — leg
lgl — lung lamella
lm — labrum

malp — Malpighian vessel
mcl — muscle
mes — mesoderm
mesbl — mesoentoblast
mesent — mesoentoderm
mg — mid-gut
mth — mouth

nc — nerve cord

oes — oesophagus

pcb — protocerebrum
pdp — pedipalp
pers — pericardial sinus
pgl — poison gland
phy — pharynx
pleye — posterior lateral eye
pmeye — posterior median eye
pp — periplasm
proct — proctodaeum
pvp — proventricular pump

ret — retina

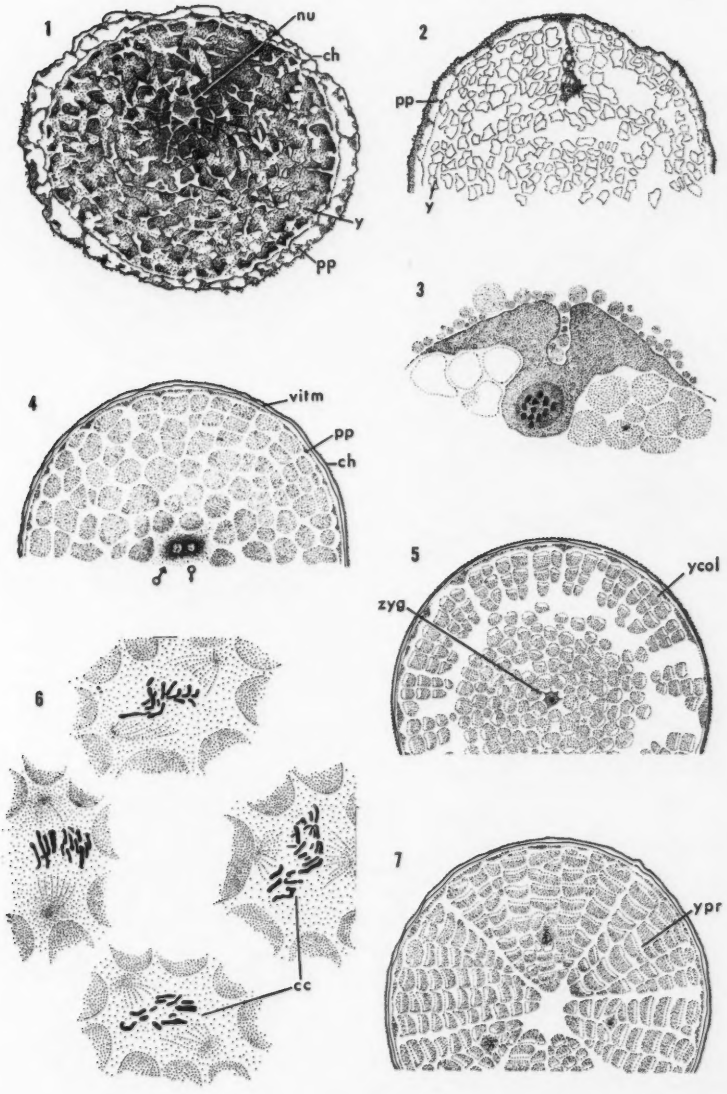
sgl — silk gland
somm — somatic mesoderm
splm — splanchnic mesoderm
spn — spinneret
spr — sperm
spt — septum
ster — sternum
stom — stomodaeum
sulv — ventral sulcus

tel — telson
tsp — tracheal spiracle

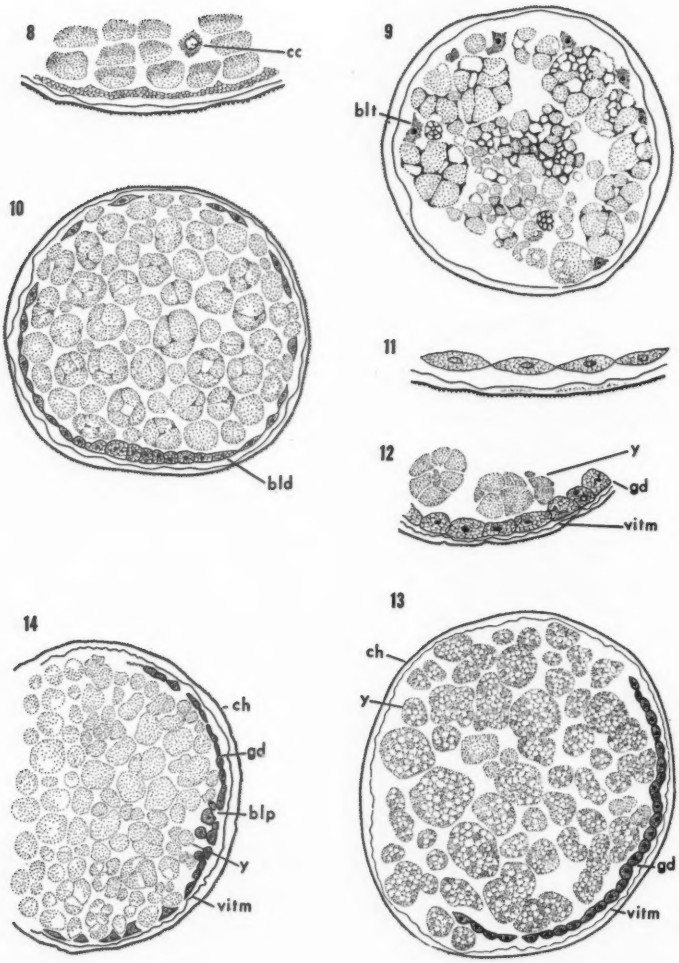
vitm — vitelline membrane

y — yolk
yc — vitellocyte
ycol — yolk column
ysac — yolk sac
ypr — yolk pyramid

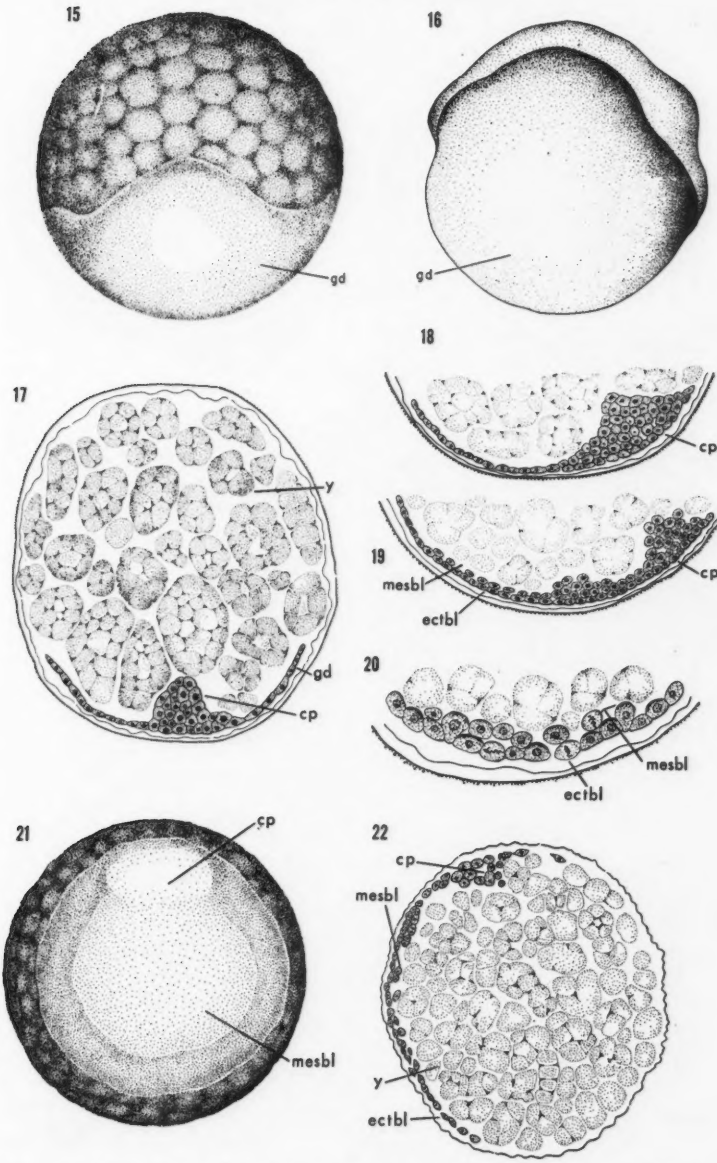
zyg — zygote



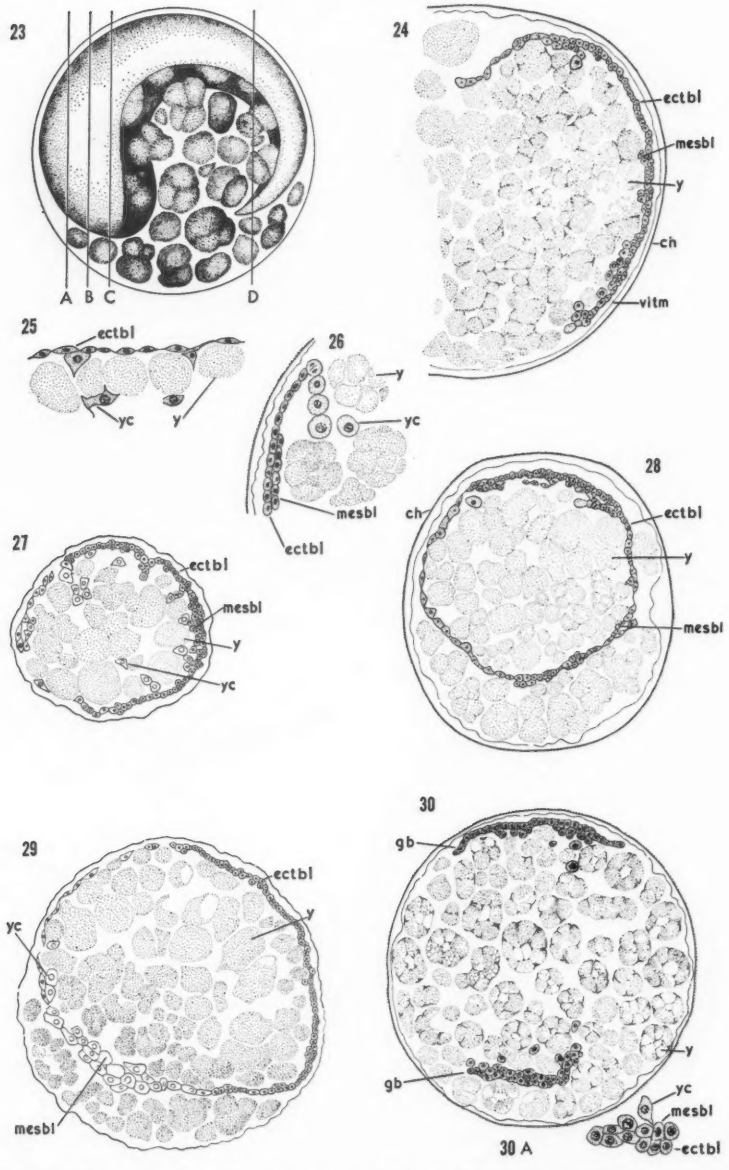
FIGS. 1–7. FIG. 1. Newly-laid egg of *Latrodectus mactans*. Section showing chorion, periplasm, yolk, and female nucleus. FIG. 2. Egg 10 minutes after oviposition, nucleus moving toward periphery. FIG. 3. First maturation division, 12 minutes. FIG. 4. Egg 4 hours old, showing male and female pronuclei just prior to fertilization. Fig. 5. Zygote, peripheral yolk in columns. FIG. 6. Second cleavage division, with nuclei in metaphase stage. FIG. 7. Eight-celled stage, with yolk columns forming pyramids.



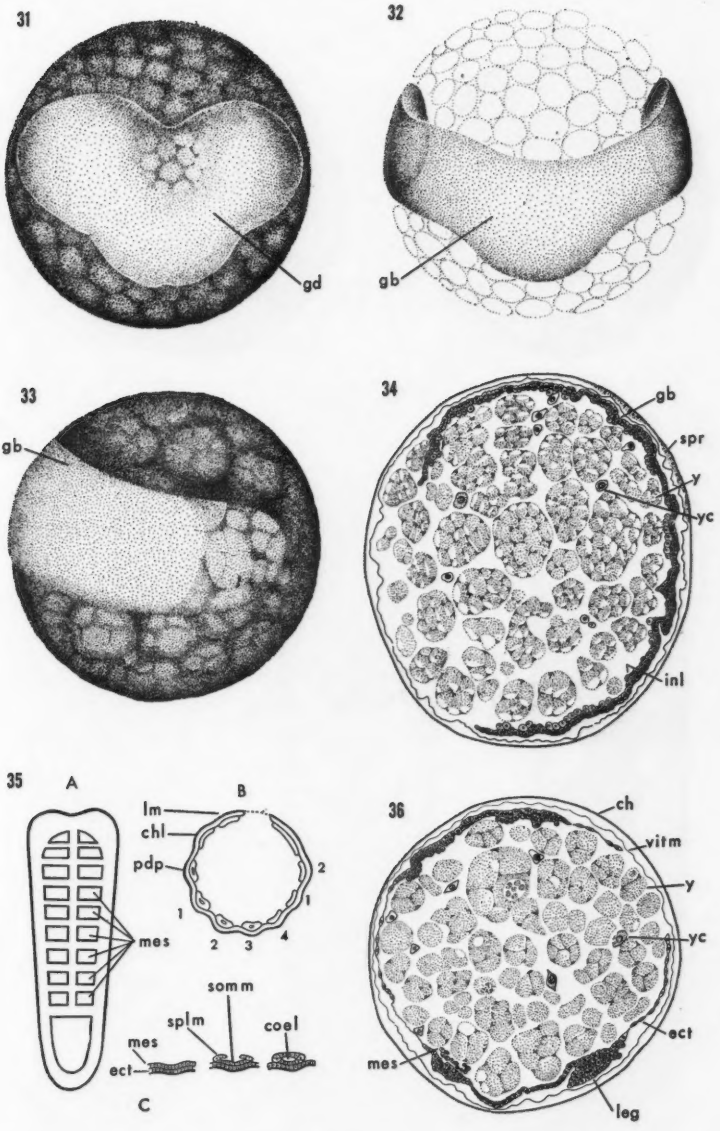
FIGS. 8–14. FIG. 8. Cleavage cell approaching periphery to enter periplasm. FIG. 9. Blastema, at 25 hours. FIG. 10. Blastoderm, at 30 hours. FIG. 11. Flattened cells of blastoderm, enlarged. FIG. 12. Blastoderm cells in region of germ disk, at 30 hours. FIG. 13. Germ disk in section, *ca.* 50 hours. FIG. 14. Germ disk at the beginning of gastrulation, 50 hours.



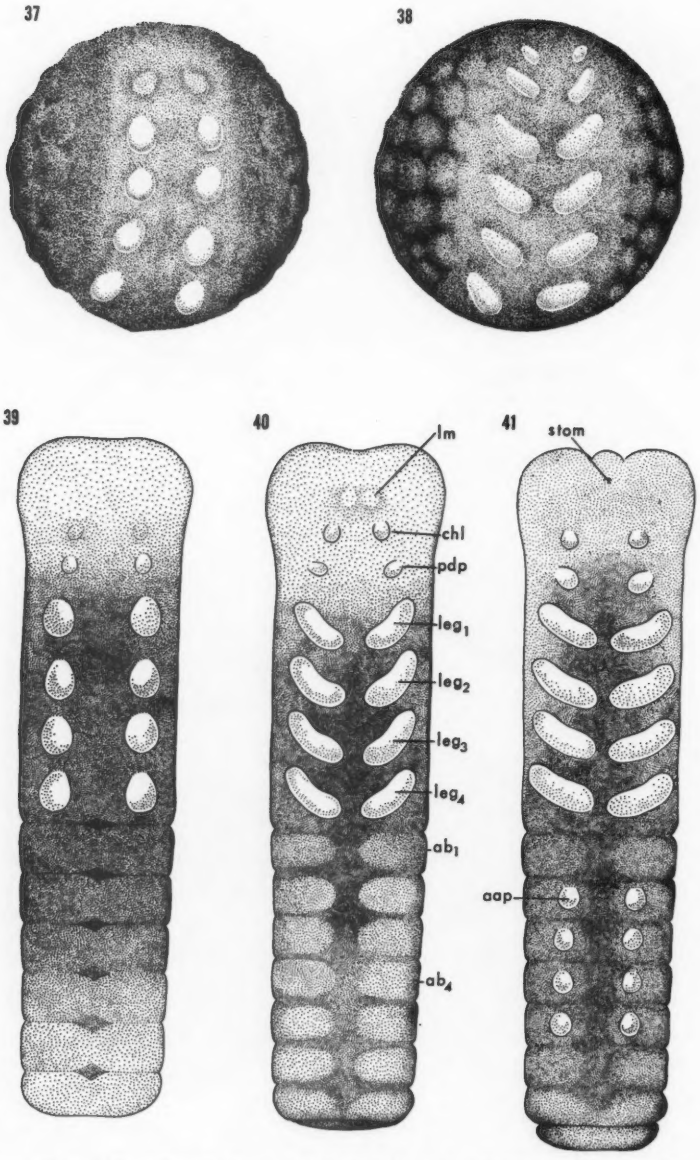
FIGS. 15–22. FIG. 15. Germ disk at 50 hours, surface view. FIG. 16. Germ disk at 50 hours, with yolk removed. FIG. 17. Germ disk at 57 hours, with primitive cumulus, in cross section. FIG. 18. As above, longitudinal section. FIG. 19. Germ disk at 90 hours, primitive cumulus shifting dorsad. FIG. 20. Germ disk at 110–115 hours, showing dual origin of cells of inner layer, greatly enlarged. FIG. 21. Surface view of germ disk at 100 hours with underlying primitive cumulus and inner layer, or mesoentoblast. FIG. 22. As above, in section.



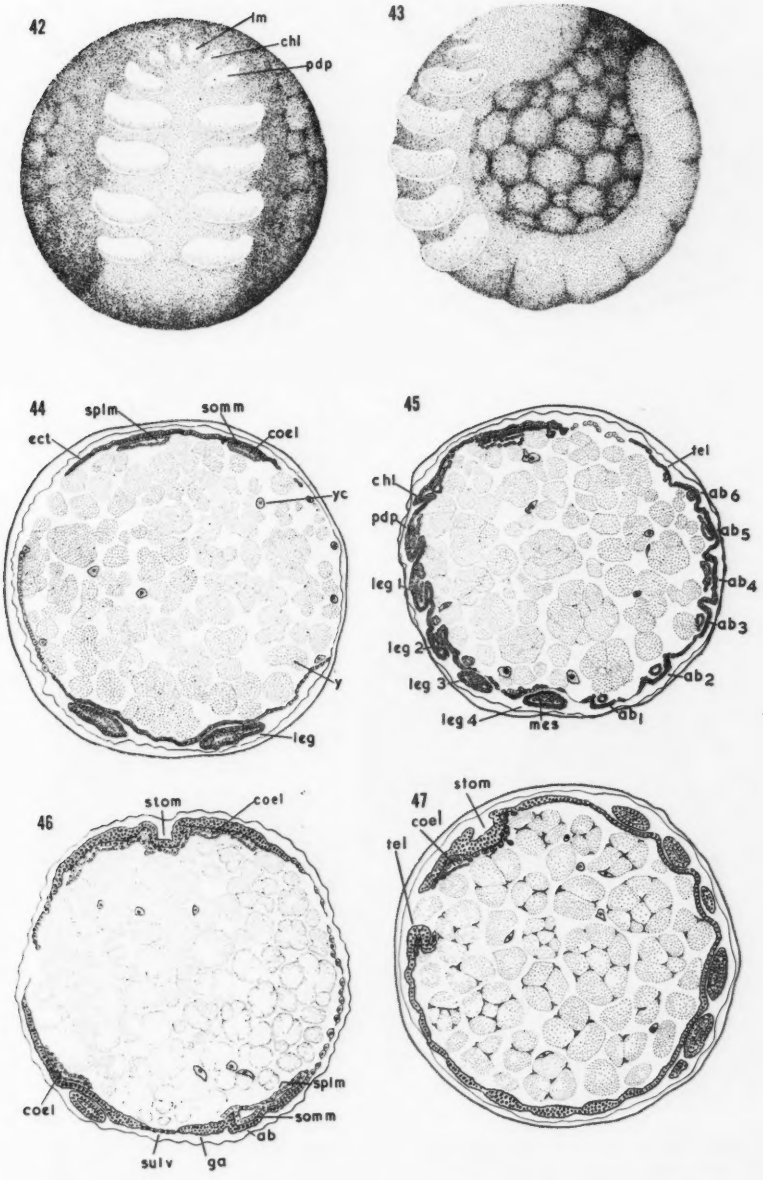
FIGS. 23–30A. FIG. 23. Diagram of germ disk at 160 hours to indicate position of sections of figures given below. FIG. 24. Sagittal section of 160-hour stage. FIG. 25. Section of germ disk to show formation of vitellocytes. FIG. 26. Section showing formation of vitellocytes from edge of germ disk. FIG. 27. Cross section of germ disk along 'A' of Fig. 23. FIG. 28. Cross section of germ disk along 'B' of Fig. 23. FIG. 29. Cross section of germ disk along 'C' of Fig. 23. FIG. 30. Cross section of germ disk along 'D' of Fig. 23. FIG. 30A. Small section of germ disk to show formation of vitellocytes from cells of inner layer.



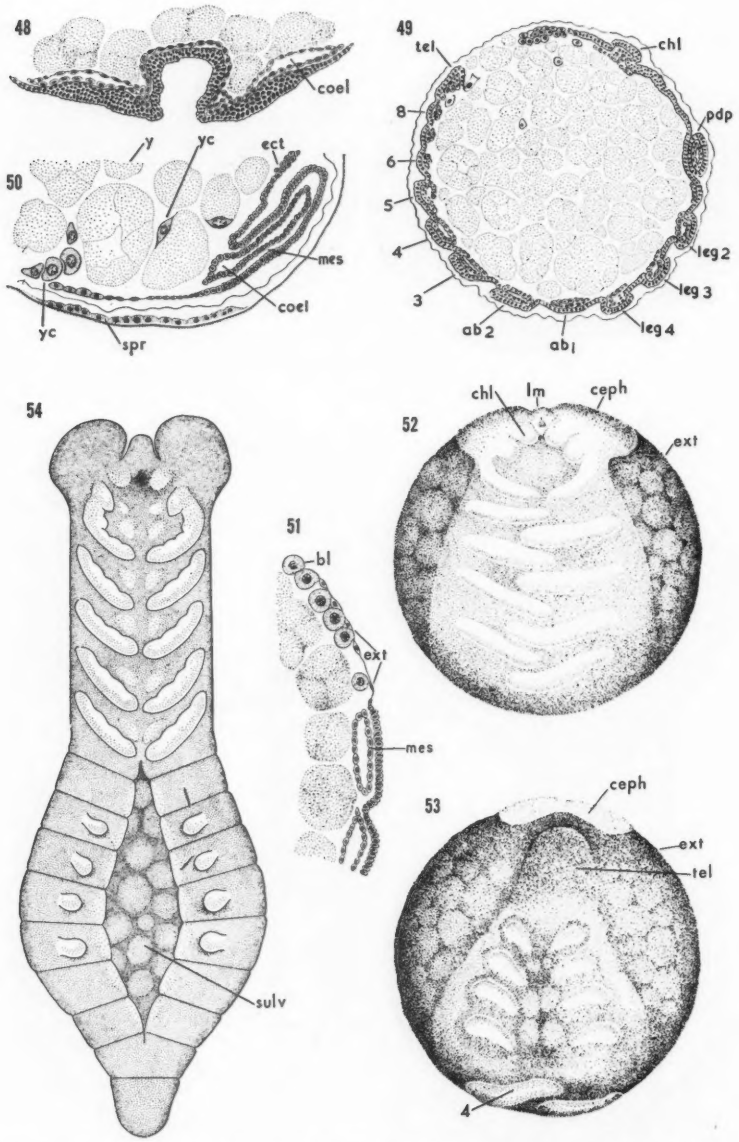
FIGS. 31–36. FIG. 31. Germ disk, surface view, 150 hours. FIG. 32. Germ band, with yolk removed, 160 hours. FIG. 33. Germ band, surface view, 160 hours. FIG. 34. Germ band, longitudinal section, 190 hours. Segmentation has set in. FIG. 35A. Diagram of 200-hour germ band showing mesodermal somites as seen in a dorsal view. Germ band flattened out. FIG. 35B. Germ band, 235 hours, seen in side view. FIG. 35C. Mode of origin of coelomic sacs. (After Montgomery.) FIG. 36. Cross section of 200-hour embryo.



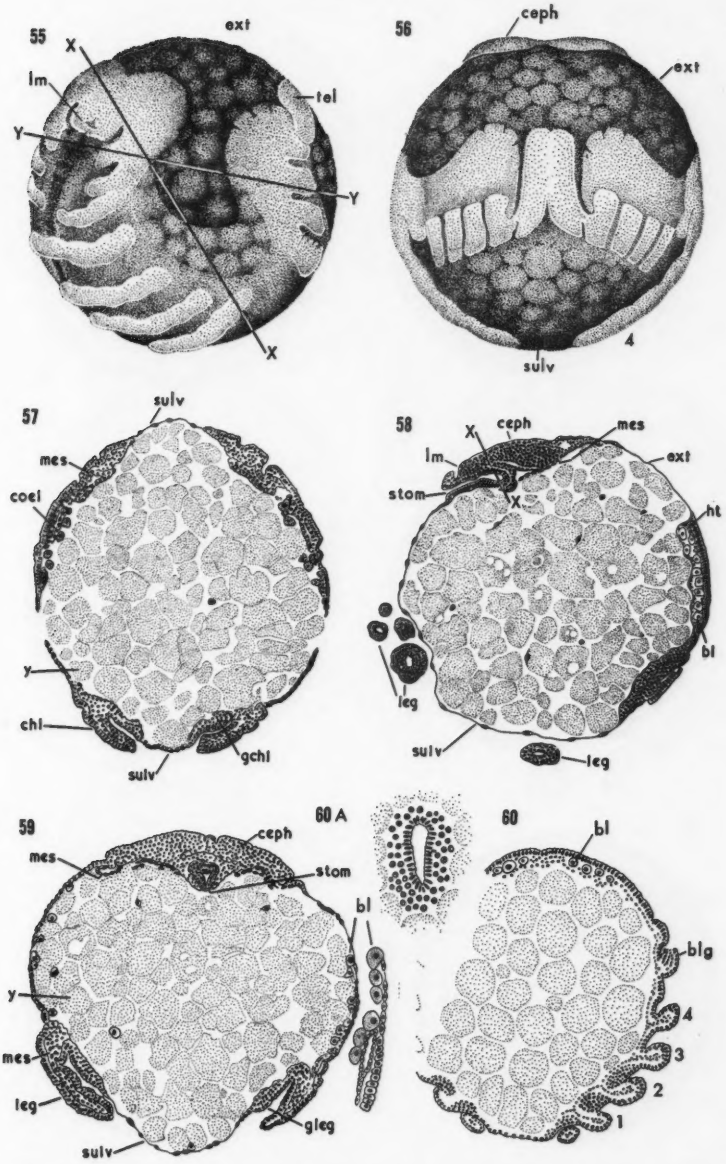
FIGS. 37–41. FIG. 37. Embryo with limb buds and pedipalps, 230 hours. FIG. 38. Embryo with limb buds, pedipalps, and chelicerae, 240 hours. FIG. 39. Diagram of 230-hour embryo, flattened out. FIG. 40. Diagram of 240-hour embryo, flattened out. FIG. 41. Diagram of 270-hour embryo, flattened out.



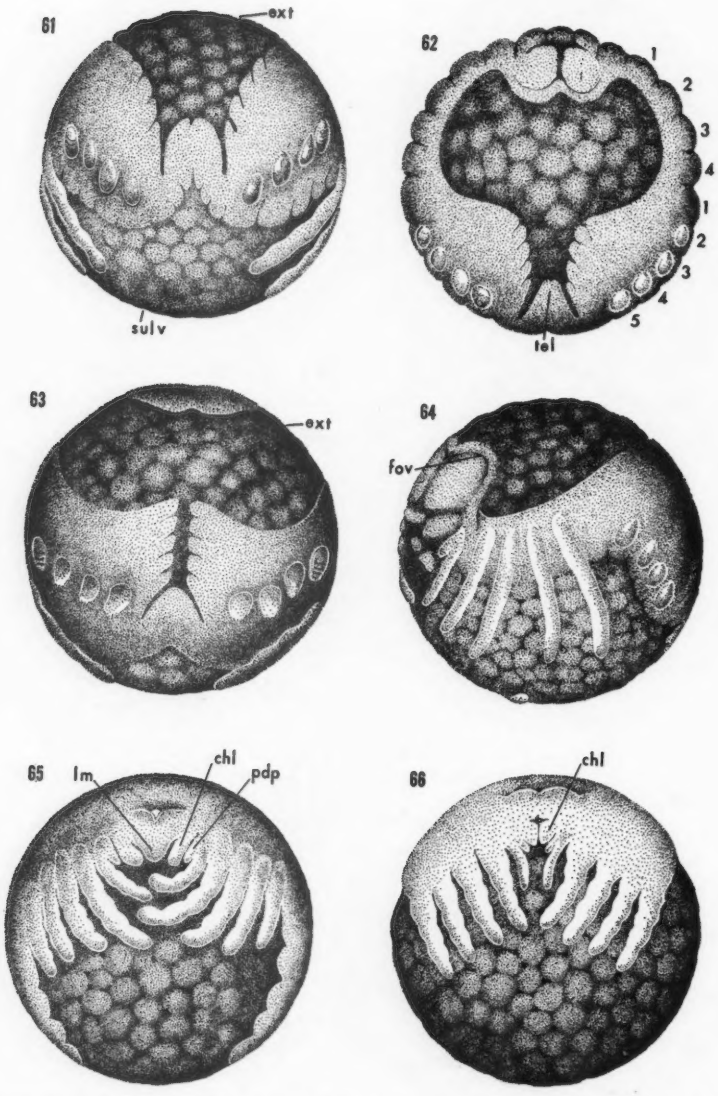
FIGS. 42–47. FIG. 42. Embryo, 250 hours, ventral view. FIG. 43. Embryo, 250 hours, lateral view. FIG. 44. As above, cross section. FIG. 45. As above, parasagittal section. FIG. 46. Embryo, 290 hours, cross section. FIG. 47. Embryo, 290 hours, sagittal section.



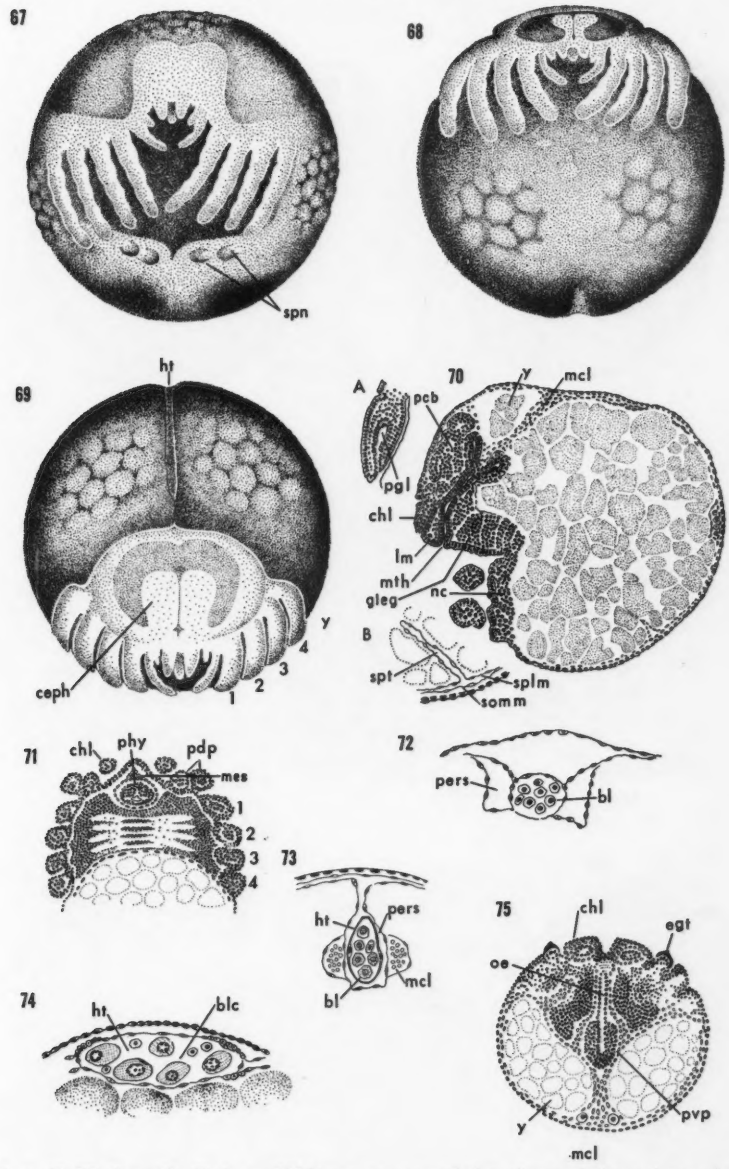
FIGS. 48–54. FIG. 48. Enlarged section through stomodaeum and rostral ganglia of 290-hour embryo. FIG. 49. Parasagittal section of 290-hour embryo. FIG. 50. Enlarged section of lateral wall of 250-hour embryo to show coelomic sac, development of vitellocytes, and polyspermy. FIG. 51. Enlarged section of extraembryonic area of 290-hour embryo to show origin of blood cells. FIG. 52. 290-hour embryo, surface view of cephalothorax. FIG. 53. 290-hour embryo, surface view of abdomen. FIG. 54. Embryo at 300 hours to show beginning of reversion.



FIGS. 55–60A. FIG. 55. Embryo, 330 hours, lateral view, to show reversion. FIG. 56. As above, posterior view. FIG. 57. As above, cross section through chelicerae, along plane Y of Fig. 55. FIG. 58. As above, sagittal section. FIG. 59. As above, cross section through stomodaeum, along plane X of Fig. 55. FIG. 60. Hortizontal section of 330-hour embryo to show appendages and primordium of book lung. FIG. 60A. Cross section through stomodaeum, along plane X of Fig. 58.



FIGS. 61–66. FIG. 61. Surface view of 340-hour embryo, posterior view. FIG. 62. As above, dorsal view. FIG. 63. Surface view of 345-hour embryo, posterodorsal view. FIG. 64. As above, lateral view. FIG. 65. Front surface view of 350-hour embryo. FIG. 66. Front surface view of 360-hour embryo to show preoral position of chelicerae and completion of first phase of reversion.



FIGS. 67–75. FIG. 67. Embryo, 365 hours, near completion of second phase of reversion. FIG. 68. Embryo, 370 hours, ventral view, reversion completed. FIG. 69. As above, dorsal view. FIG. 70. As above, sagittal section of 370-hour embryo. FIG. 70A. As above, section of chelicera to show primordium of poison gland. FIG. 70B. As above, to show abdominal septum enlarged. FIG. 71. Horizontal section of cephalothorax of 370-hour embryo. FIG. 72. Diagram to show formation of heart in 370-hour embryo. FIG. 73. Diagram to show completed heart in pericardium in 380-hour embryo. FIG. 74. Longitudinal section of heart in 380-hour embryo to show variation in size of blood cells. FIG. 75. Cross section of cephalothorax of 370-hour embryo.

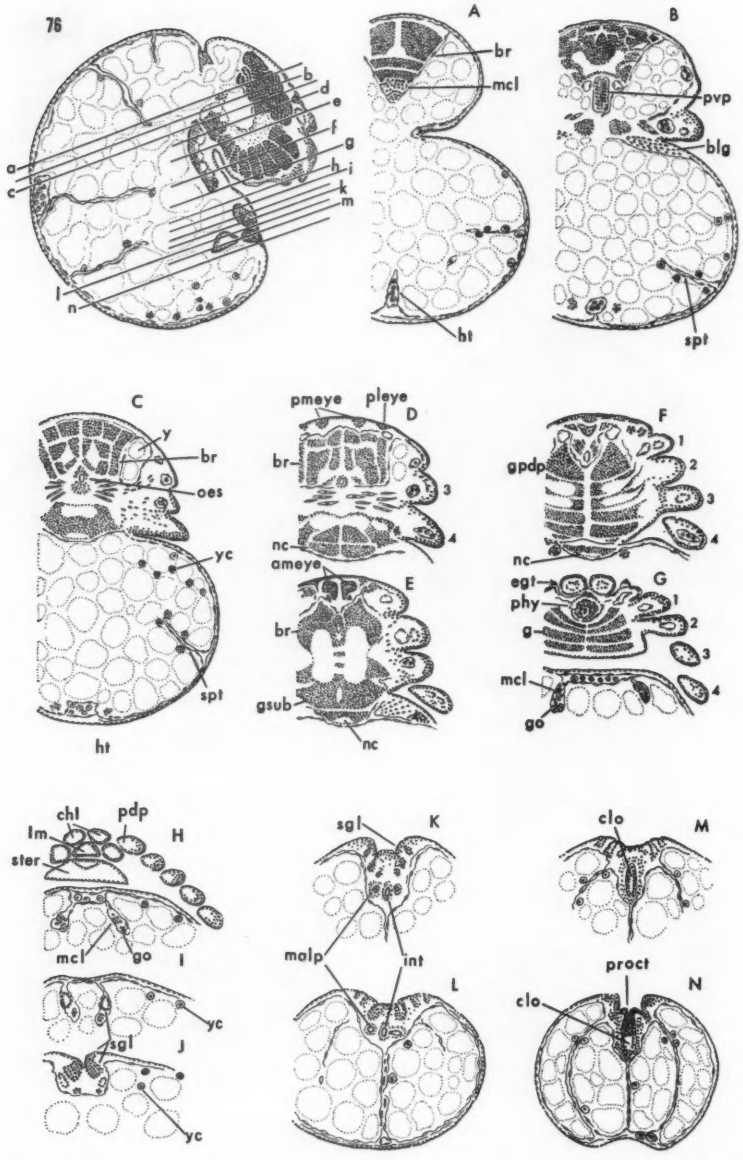
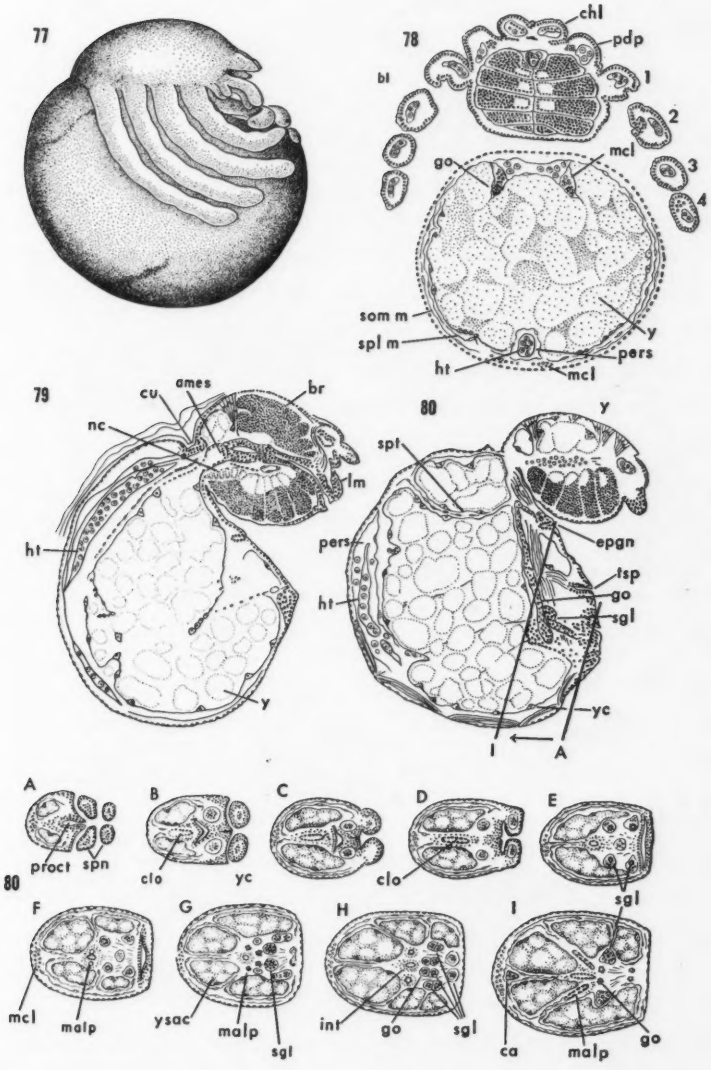
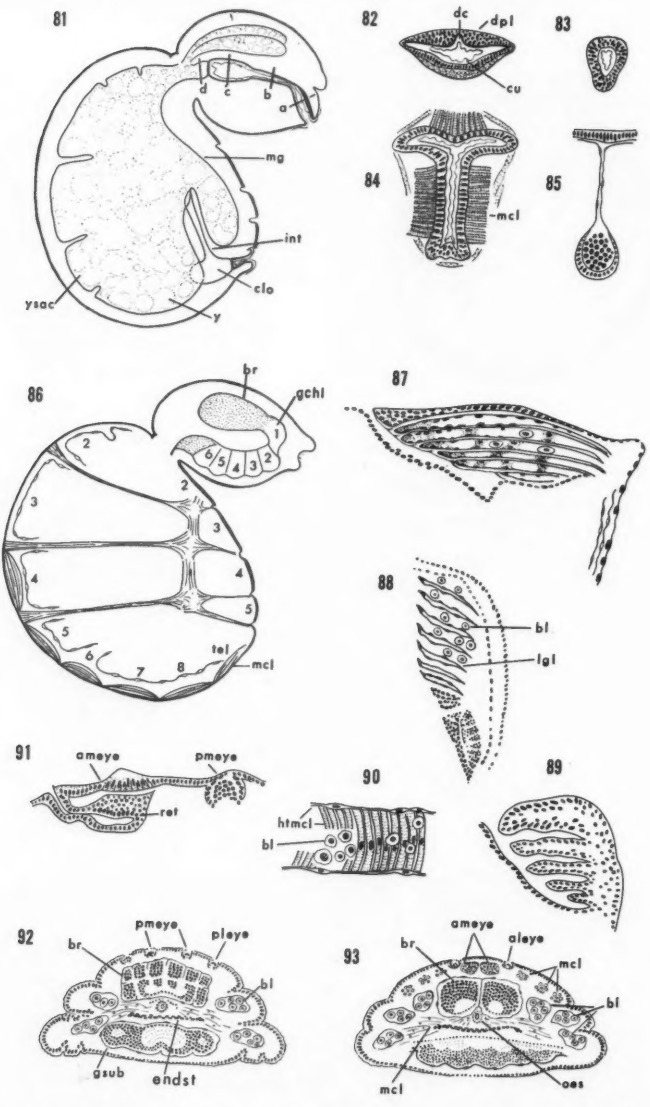


FIG. 76. Parasagittal section of 380-hour embryo to show position of cross sections, A to N.



FIGS. 77–80 I. FIG. 77. Embryo ready to emerge, 400 hours, surface view. FIG. 78. As above, horizontal section. FIG. 79. As above, sagittal section. FIG. 80. As above, parasagittal section. FIGS. 80 A–I. As above, cross sections, through planes indicated in Fig. 80.



FIGS. 81–93. FIG. 81. Diagram of digestive system in embryo ready to emerge. FIG. 82. Cross section through pharynx, at *a* of Fig. 81. FIG. 83. Cross section through oesophagus, at *b* of Fig. 81. FIG. 84. Cross section through proventricular pump, at *c* of Fig. 81. FIG. 85. Cross section through anterior region of mesenteron, at *d* of Fig. 81. FIG. 86. Diagram of 400-hour embryo with nerve ganglia and muscles to show segmentation. FIG. 87. Book lung in 400-hour embryo, sagittal section. FIG. 88. Book lung in 400-hour embryo, horizontal section. FIG. 89. Book lung in 320-hour embryo. FIG. 90. 400-hour embryo, showing heart muscle. FIG. 91. As above, sagittal section through anterior and posterior median eyes. FIG. 92. As above, cross section of cephalothorax, through posterior median and lateral eyes. FIG. 93. As above, cross section through anterior median and lateral eyes.

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THE MORPHOLOGY OF THE NEMATODE RADOPHOLUS GRACILIS (DE MAN, 1880) HIRSCHMANN, 1955, PARASITIC IN ROOTS OF WILD RICE, ZIZANIA AQUATICA L.[1]

K. C. SANWAL[2]

Abstract

The anatomy of *Radopholus gracilis* is described, the description being based chiefly on the study of living specimens and intravital staining with methylene blue and neutral red. Cajal's silver impregnation method and Golgi's bichromate silver method, both with slight modifications, prove useful.

Cuticle striated with four longitudinal incisures; 16–18 muscle cells in anterior region; head supported by six-ridged cuticular framework with six lips and 16 sense organs; excretory system with anterior and posterior canals opening into an ampulla, all on left side of the body, and a cuticular terminal duct; ovaries opposed and outstretched; vas deferens differentiated into three regions and no central rachis in the gonads; nerve ring associated with a group of 7, 13, and 27 cells on dorsal, ventral, and lateral sides respectively; phasmid nerve supply from lateral nerves; 'hemizonid' organ present. The nematodes appear to be adapted to an aquatic environment and seem to be unaffected by low temperatures and low oxygen content in their habitat.

Introduction

This paper deals with some observations on the anatomy of *Radopholus gracilis* (de Man, 1880) Hirschmann, 1955 (5), which has been frequently reported infesting the roots of a large number of plants in different parts of the world. The specimens used for this work were recovered from the roots of wild rice (*Zizania aquatica* L.) in Ontario, Canada. The study was undertaken at the suggestion of Dr. A. D. Baker, primarily to obtain a good anatomical description of one of the species of *Radopholus*, a nematode genus which is of great economic importance in view of the fact that its various species parasitize a large number of plants some of which are important food crops like the cultivated rice. Secondly, studies of the morphology of this species may prove helpful in understanding the general structure and relationships of the various nematodes belonging to the superfamily Tylenchoidea, a nemic group of great economic importance.

Materials and Methods

Live specimens of *R. gracilis* were obtained from roots of wild rice growing on the banks of the Jock River in the vicinity of Richmond, Ontario. The roots, after being coarsely washed under a tap to remove the adhering soil, were placed upon a 150 mesh sieve on a Baermann funnel and the worms were tapped off at the bottom after 24 hours.

[1]Manuscript received October 11, 1956.
Contribution from Nematology Section, Entomology Laboratory, Science Service, Department of Agriculture, Ottawa.
[2]Postdoctorate Fellow, National Research Council of Canada.

The major part of the anatomy was studied in the living state using methylene blue and neutral red as intravital stains. The nematodes were left in a lightly tinted aqueous solution of these dyes and were examined from time to time. Although they could live normally in the stain for more than 48 hours, they were best suited for study after remaining in the solution for 10–15 minutes. During this time the reproductive system and the oesophageal glands were sufficiently stained; later the stain gives rise to vacuole-like structures in the body and the organs start losing color. The blue color imparted by methylene blue can, however, be fixed by treating the stained worms with a solution of picrocarmine for about 2 minutes and then transferring them to a mixture of 70% alcohol and glycerin (100 : 1). After the alcohol has evaporated the worms are mounted in anhydrous glycerin and sealed with 'zut'. Ammonium picrate does not give good results in fixation of methylene blue stain.

For the study of cuticular striations, nervous system, reproductive system, amphids, and phasmids, very good results were obtained by staining with silver by Cajal's method (3) with slight modifications. Living worms were placed in a small vial containing a mixture of 2% silver nitrate solution and absolute alcohol mixed in the ratio of 10 : 1. The solution was agitated vigorously by blowing in air with a finely drawn pipette. This served to straighten the worms. The vial was then tightly corked, wrapped in black paper, and placed in an oven at a temperature of 35° C. for 48 hours. The specimens were then washed with distilled water for about 2 minutes and transferred to a mixture of hydroquinone (2 g.), formalin (10 cc.), and distilled water (100 cc.). The reduction of silver nitrate was watched under the binocular microscope. After the worms had attained a dark brown coloration, they were transferred to the glycerin–alcohol mixture which had been very feebly acidulated with formic acid. They were left in this mixture in an open watch glass until the alcohol evaporated. This was best achieved by leaving the watch glass in an oven at a temperature of 30° C. for about 12 hours. The nematodes were finally mounted in anhydrous glycerin, acidulated feebly with a drop of formic acid. The slides were ringed with 'zut'.

Alternatively, Golgi's bichromate silver method (3) gave the same results. Living worms were fixed in a mixture of 1% osmic acid and 3% potassium bichromate (3 : 1), and were left in a corked vial for 72 hours. They were then left in a 2% solution of silver nitrate for about 8 hours, after which they were transferred to a very small quantity of glycerin–alcohol mixture and placed in an open watch glass in the oven at a temperature of 30° C. until the alcohol evaporated. The worms were finally mounted in anhydrous glycerin feebly acidulated with formic acid. This method gave very good details of cuticular striations, lateral incisures, phasmids, and amphids (Figs. 31, 33, 34). The slides prepared by the above two methods can be kept for a period of 6–8 weeks, after which the specimens begin to lose transparency and start showing some fine granular condensation on their surfaces.

For sectioning, the worms were fixed in aqueous Bouin and after being washed with 70% alcohol were imbedded in 2% agar block in the manner described by Mulvey (6). The agar block was dehydrated in alcohol, cleared in xylol, and imbedded in Fisher tissuemat. Sections were stained with eosin and haemotoxylin. Equally good results were obtained by using Gray's double stain and celestian B.

General Description

Dimensions.—Female: length 1.6–2.6 mm.; a = 45.7–65.6; b = 4.3–6.1; c = 13.2–20.8; v = 45.7–62.5%; stylet 0.018–0.025 mm.; excretory pore 0.135–0.19 mm. from anterior end of the body; number of worms measured 17. *Male:* length 1.52–2.13 mm.; a = 44.7–66.07; b = 4.2–5.1; c = 13.2–22.6; spicules 0.031–0.032 mm.; stylet 0.015–0.019 mm.; excretory pore 0.13–0.016 mm. from the anterior end of the body; number of worms measured 10.

Body is long, slender, thread-like in shape, appearing white in color to the naked eye but transparent with a greenish tint under a microscope. Body diameter is almost uniform from the level of the oesophageal bulb to the anus, gradually narrowing from these points towards the two extremities (Figs. 1, 2). Tail with a finely pointed terminal process (Figs. 1, 2, 11) in both sexes. This, however, does not seem to be a specific character, there being variations from a bluntly tipped tail to a tail with a fine terminal tip (Fig. 5). Cuticle is transversely striated and also bears four longitudinal incisures. The cuticle of the tail region of the male forms the caudal alae (Figs. 2, 11).

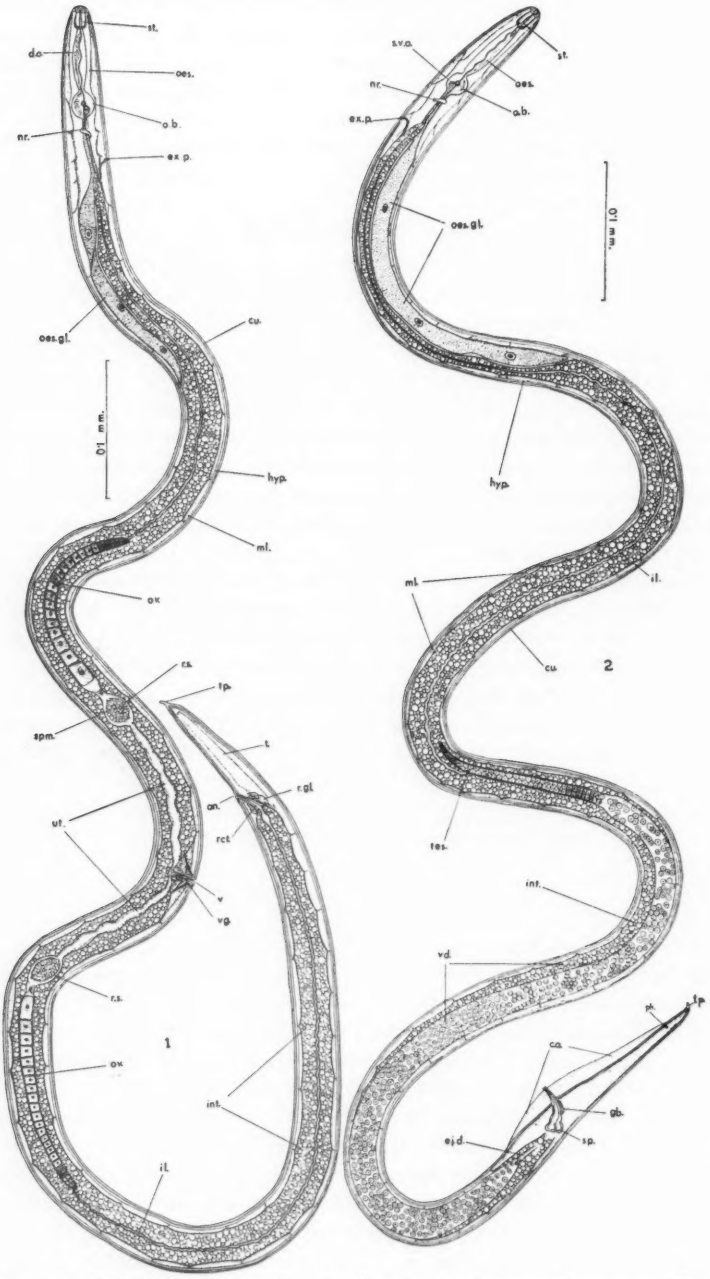
The stylet (Fig. 3) is stout and knobbed at the base. Oesophagus is long with a median bulb (Figs. 1, 2, 6, and 10) containing a refractive bean-shaped valve. The oesophageal glands separate from the oesophagus prior to its junction with the intestine (Figs. 1, 2, 6, 10) and hang freely into the body cavity lying parallel to the intestine. The intestine, which has a narrow lumen, runs to near the posterior end of the body where it joins the short, narrow, and cuticularized rectum.

The two ovaries in the female are opposed and outstretched leading to the two uteri, which join together to open outside at the vulva through a short vagina with cuticularized walls. The vulva is situated slightly behind the middle of the body on the midventral line. A receptaculum seminis is interposed between the ovary and the uterus of each side (Figs. 1, 21).

The single testis in the male extends forward slightly beyond the middle of the body and is continuous with the wide vas deferens. The vas deferens empties into the cloaca through a short and muscular ejaculatory duct (Figs. 2, 15, 30). The two spicules are equal and symmetrically placed. The gubernaculum is plough-shaped (Figs. 2 and 13).

The nerve ring surrounds the oesophagus almost midway between the oesophageal bulb and the beginning of the intestine in both sexes (Figs. 1, 2).

The excretory pore is situated on the midventral line, generally slightly posterior to the nerve ring but its position is slightly variable in both sexes (Figs. 1, 2, 6, 7).



FIGS. 1–2. *Radopholus gracilis*. FIG. 1. Female, showing general anatomy. FIG. 2. Male, showing general anatomy.

Cuticle and Hypodermis

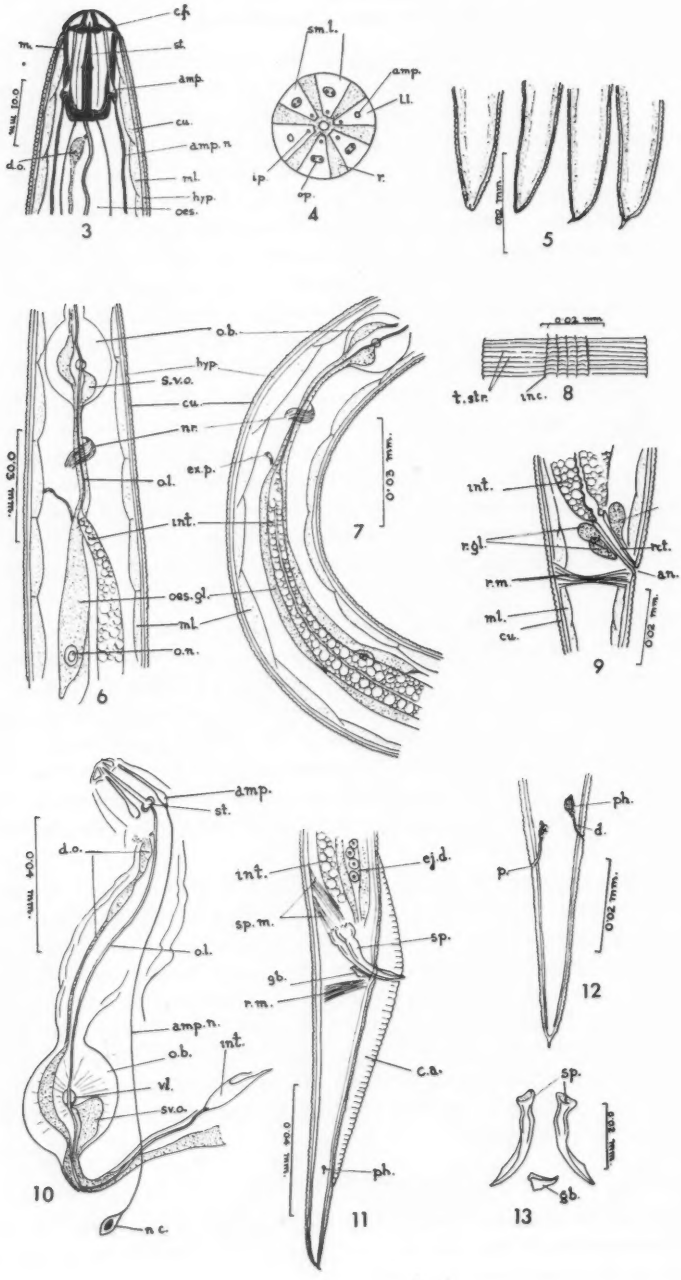
The body is covered by a thick and tough cuticle which is continuous with the cuticular lining of the rectum and vagina of the female and the cloaca of the male. The cuticle shows distinct transverse striations in both sexes. The transverse striations on the general surface of the body are 0.0015 mm. apart in the female and 0.0013 mm. apart in the male. The striations on the caudal alae of the male are slightly wider apart than those on the general surface of the body. There are four–five transverse striations on the cuticle covering the head region in both sexes and these are much narrower than those on the general surface of the body. Besides these transverse striations there are four longitudinal incisures on the cuticle which run almost the entire length on the two lateral sides of the body. These lateral incisures run parallel to each other and occupy about one-fourth the diameter of the body, except in the postanal region of the female and the caudal region in the male. In the postanal region of the female the incisures draw nearer to each other, the two middle ones finally converging and the two outer ones reaching almost the tip of the tail. In the male the two incisures towards the dorsal side of the body extend almost to the tip of the tail, while the two ventral ones widen and gradually disappear after running for some distance along the caudal alae. The transverse striations of the cuticle extend through these lateral fields (Fig. 8).

The hypodermis is thickened along the median lines and the lateral lines to form the four longitudinal chords. These are very poorly developed in the anterior region of the body. There is, however, a circular band of tissue surrounding the buccal chamber in the anterior region of the body (Fig. 35) through which probably pass the nerves to the cephalic papillae as indicated by the 10 nuclei seen in the tissue in a cross section (Fig. 35). This circular band of tissue might probably correspond to the 'arcade' described in other forms (1). Well defined cells of the arcade, as have been described for the other forms, could not be observed.

All the four longitudinal chords are very poorly developed in the anterior region of the body. It is at the level of the oesophageal bulb that they become apparent (Fig. 36). Posterior to this point, although the dorsal and ventral chords remain almost unchanged in size, the lateral chords gradually enlarge and become wide and voluminous, occupying all the lateral margin and part of the laterodorsal and lateroventral margins of the body, pushing the muscular quadrants into the dorsolateral and ventrolateral margins (Figs. 38 and 39). The dorsal and ventral chords diminish in size in the postanal region of the body till they disappear almost completely, leaving only the wide and voluminous lateral chords.

Musculature

Beneath the hypodermis is disposed the muscular layer, which is divided into four quadrants by the four longitudinal chords. In a cross section of the anterior region of the body where the longitudinal chords are inconspicuous,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there are 16–18 muscle cells disposed around the periphery (Fig. 35). In the region of the body near the muscular bulb (Fig. 36) there are five–six muscle cells in each quadrant and the longitudinal chords become apparent. In the region of the nerve ring (Fig. 37) six–seven muscle cells are seen in each quadrant and the lateral chords start widening. At the level of the blind end of the ovary in the female and in the middle region of the body in the male seven–eight muscle cells are seen in each quadrant. Towards the posterior extremity of the body the muscle cells gradually diminish in number till there are only two–three muscle cells left, which are confined to the dorsal and ventral sides of the body (Fig. 40), and these too finally disappear in the vicinity of the phasmids.

In addition to the muscular layer of the body wall, there are special sets of muscles associated with the stylet, vulva, spicules, anus, and the cloaca, which will be referred to with the description of the individual organ systems.

Head and Lips

The term 'head' is applied to the anterior region of the body which is supported by a cuticular framework and bears the lips. The head is rounded and measures 0.009–0.01 mm. in diameter in the male, and 0.009–0.013 mm. in the female. It is marked off from the rest of the body by a slight constriction which can be observed better by mounting a specimen in Canada balsam without soaking it in a mixture of balsam and xylol. This serves to make the specimen opaque, thus allowing a clearer observation of the contours of the body. The cuticle of the head region bears four–five transverse striations.

The head is supported by a cuticular framework which gives the appearance, on casual observation, of being made up of separate pieces. It seems to me that the whole framework is one piece and is formed by a heavy local sclerotization in the tissue of the head region. It consists of six ridges placed radially around the central oral aperture, with their outer basal portions joined together to form a circular piece surrounding the base of the lips. The inner ends of these ridges converge to meet along their inner borders to form a guide for the stylet, and, at their tips, to form a circular oral disk around the oral aperture. On an en face view of the head (Fig. 4), the six ridges appear as the spokes of a wheel with the oral aperture as the axle.

There are six lips, two lateral and four submedian, separated from each other by the six cuticular ridges (Fig. 4). Each of the six lips bears a minute papilla close to the oral aperture. The four submedian lips have in addition two minute papillae on each lip. These two papillae on each submedian lip seem to be so near to each other that each pair gives the appearance of a single

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FIGS. 3–13. *Radopholus gracilis*. FIG. 3. Anterior region of female. FIG. 4. Head of female, end on view. FIG. 5. Variation of the female tail. FIG. 6. Oesophageal region of the male. FIG. 7. Oesophageal region of young female. FIG. 8. Transverse striations and lateral incisures of the cuticle. FIG. 9. Anal region of the female. FIG. 10. Oesophageal region of the female. FIG. 11. Tail region of the male. FIG. 12. Tail region of female showing phasmids. FIG. 13. Spicules of male with gubernaculum.

large papilla. The lateral lips carry the amphidial pores. Thus there are 16 cephalic sense organs in all placed on the six lips; six papillae in the inner circle, eight papillae in the outer circle, and two amphidial apertures (Fig. 4).

Excretory System

The excretory pore is situated on the midventral line of the body slightly behind the level of the nerve ring (Figs. 1, 2). It leads into the long terminal excretory duct, the walls of which are cuticular and highly refractive in nature. Immediately behind the excretory pore this terminal excretory duct is inflated to form a beak-like structure (Fig. 17). The duct runs for a short distance towards the middle of the body where it enters the lateral chord and then follows a posterior course, lying imbedded in the left lateral chord. In the living worm the duct follows a smoothly wavy course with its cuticular walls of uniform thickness and its lumen showing a uniform diameter (Fig. 16). Immediately after the death of the worm, however, the duct assumes a perfectly straight course and its walls show alternating regions of thickness and thinness, thereby changing the diameter of its lumen (Fig. 17). Thus a dead worm gives the impression that the cuticular walls of the duct and its lumen are not uniform although this is not the case.

At its inner end the lumen of the terminal excretory duct widens slightly but a renette cell, as figured by Cobb (2) in *Radopholus similis*, could not be made out. There is, however, a brightly refractive spot near the termination of the duct which might represent the nucleus of the renette cell. It is also very difficult to say if this cuticular terminal excretory duct has any protoplasmic covering, although brightly refractive spots resembling small nuclei are seen along the two sides of the cuticular wall of the duct. There is, however, a definite indication of protoplasmic covering towards the inner end of the duct, as the area around this extremity gets lightly stained with intravital stains and shows a refractive spot situated very near to the extremity of the terminal duct (Fig. 16).

Using methylene blue as an intravital stain, the following system of excretory canals was observed. This system, however, is rather difficult to observe and is very likely to be overlooked on casual examination. It becomes totally obliterated in dead or fixed specimens. It shows best in adult living specimens. In the younger males and females the system is present but is more difficult to observe. It can be seen by rotating the living worm under the cover slip with a slight push of the fingers. It consists of two long tubes which do not have any cuticular lining or a protoplasmic covering. The tubes, hereafter termed 'canals', appear like closed narrow channels in the lateral chord and seem to originate from an ampulla-like structure (Fig. 16). This ampulla-like structure is situated at the inner end of the cuticular terminal excretory duct and resembles a big irregular vacuole. It is doubtful whether there is any connection between the cuticular excretory duct and the ampulla, although the cuticular excretory duct appears to open into the ampulla. From this ampulla arise the two tubular canals mentioned above, one of them

proceeding anteriorly and running parallel and close to the cuticular excretory duct, while the other proceeds posteriorly. The anterior canal continues straight towards the anterior end of the body and could be traced up to the vicinity of the basal knobs of the stylet. The posterior canal, which is much wider in diameter, follows a slightly sinuous course and could not be traced beyond the vulvar region of the body. Another interesting feature about these two canals is that they appear to enlarge into small ampulla-like swellings all along their course. These swellings take various shapes and keep changing (Fig. 18). In fact, all along the length of the canals, structures resembling ampullae and vacuoles appear and disappear continuously in the living worm. In most specimens the anterior canal shows an ampulla-like enlargement in the vicinity of the base of the stylet but the shape of this enlargement varies in different specimens. The posterior canal is seen spirally twisted at places in some specimens, or it may be straight with a perfectly uniform diameter, while in some specimens it shows ampulla-like enlargements along its course (Fig. 16).

Digestive Tract

At the anterior end of the body there is a circular aperture bounded by the six lips and the cuticular oral disk formed by the fusion of the six ridges of the head framework. This oral aperture leads into a narrow and cylindrical cavity, the wall of which appears to be formed by the apposition of the inner borders of the six ridges of the cuticular head framework. The 'buccal tube' thus formed serves as a stylet guide. The stylet, which passes through this 'buccal tube', consists of three regions: an anterior sharply conical region, a middle stout and cylindrical region, and the basal knobs (Fig. 3). The basal knobs are three in number and each knob is faintly bilobed. Two strong bands of muscle fibers are inserted between the base of the stylet and the lateral corners of the head. These muscles serve for the forward movement of the stylet (Figs. 1, 2, 3). The stylet has a narrow canal traversing through the three regions from the tip of the anterior conical region to the base of the knobs. This canal leads into the lumen of the oesophagus.

The oesophagus is divisible into three regions: the prebulbar region, the bulbar region, and the postbulbar region. The prebulbar region of the oesophagus is widest at the middle and narrows gradually towards the base of the stylet and the oesophageal bulb. The oesophageal bulb is well developed and squarishly-oval in shape in both sexes, showing radiating fibers on its walls (Figs. 1, 2, 6, 7, 10). The postbulbar region of the oesophagus is narrow and has an almost uniform diameter. The lumen of these three regions of the oesophagus is formed by a cuticularized tube which has a refractive appearance. The diameter of this cuticularized lumen is uniform throughout except inside the muscular bulb. Inside the muscular bulb two bean-shaped local thickenings of the walls of the cuticular lumen give rise to a prominent valve which is situated at the center of the muscular bulb (Figs. 1, 2, 6, 7). The lumen of the cuticularized tube within this valve is slightly wider than the lumen of the preceding and succeeding regions.

The above regions of the oesophagus are chiefly muscular. The glandular region of the oesophagus is formed by the oesophageal glands which lie imbedded in the muscular tissue of the oesophagus up to a point slightly anterior to the beginning of the intestine. At this point the glandular part (the oesophageal glands) separates from the muscular part as a tongue-like extension which gradually becomes broader as it extends posteriorly and lies parallel to the intestine (Figs. 1, 2, 6, 7, 10). The components of this glandular tongue-like extension are the three oesophageal glands, one dorsal and two subventral. These three glands appeared to be fused in a compact glandular mass with three nuclei in a stained specimen. When a living specimen is examined it appears that the distal swollen lobes of these oesophageal glands are separate from each other, all lying in a compact mass. The two subventral glands extend a long distance posteriorly, lying for the most part free in the body cavity with only their thin anterior prolongations imbedded in the muscular oesophagus. These two glands open through an ampulla into the lumen of the oesophagus at the base of the bean-shaped valve (Figs. 6, 7, 10). The free swollen lobe of the dorsal oesophageal gland lies anteriormost in the compact glandular mass. Its narrow anterior prolongation is imbedded in the postbulbar region of the oesophagus, curves around the bean-shaped valve through the tissue of the muscular bulb, and proceeds anteriorly within the prebulbar region of the oesophagus. At the anterior end of the prebulbar region of the oesophagus it enlarges into a small ampulla which discharges through a minute duct into the cuticular lumen of the oesophagus slightly behind the basal knobs of the stylet (Figs. 1, 2, 3). The oesophageal glands have a finely granular appearance. The three nuclei of the oesophageal glands lie in the posterior lobed portion of each gland. The dorsal gland appears to have a different affinity for the intravital stains used than the two subventral glands; the latter taking up the stain more readily in comparison to the former.

The lumen of the postbulbar region of the oesophagus opens into the intestine (Figs. 1, 2, 6, 7). At the junction of the intestine and the cuticular lumen of the oesophagus are interposed two–three small rectangular cells which are slightly refractive in nature and look different from the surrounding tissue. Whether these cells are a continuation of the cuticular lumen of the oesophagus or modified cells of the intestinal wall could not be ascertained.

The intestine is very narrow in diameter at its anterior extremity and here it runs parallel to the oesophageal glands (Figs. 1, 2). It widens at the level of the posterior extremity of the oesophageal glands and occupies almost all the available space in the body cavity. In the female it runs posteriorly as a straight tube of almost uniform diameter except at the vulvar region, where it narrows considerably to allow for the deep vagina (Figs. 1, 19). Posteriorly it narrows slightly in the preanal region and leads into the rectum. The rectum is short and narrow with a thick cuticular lining which is continuous with the cuticle of the body surface. The anus is situated on the midventral line (Figs. 1, 9).

In the male the intestine is much narrower in diameter as it runs alongside the male gonaduct and empties into the cloaca from the dorsal side. The cloaca, which is lined with cuticle, is very short and narrow. The lumen of the intestine is very narrow and follows a sinuous course in both sexes. The intestinal wall is densely filled with spherical fat globules that give the worm its greenish tint.

On close examination of living female worms three very obscure granular cell masses were seen on the sides of the rectum (Fig. 9). These granular cell masses may probably represent the rectal glands. Their presence is also suspected in the male worms, although they could not be observed in them because of the poor visibility of the cloacal region.

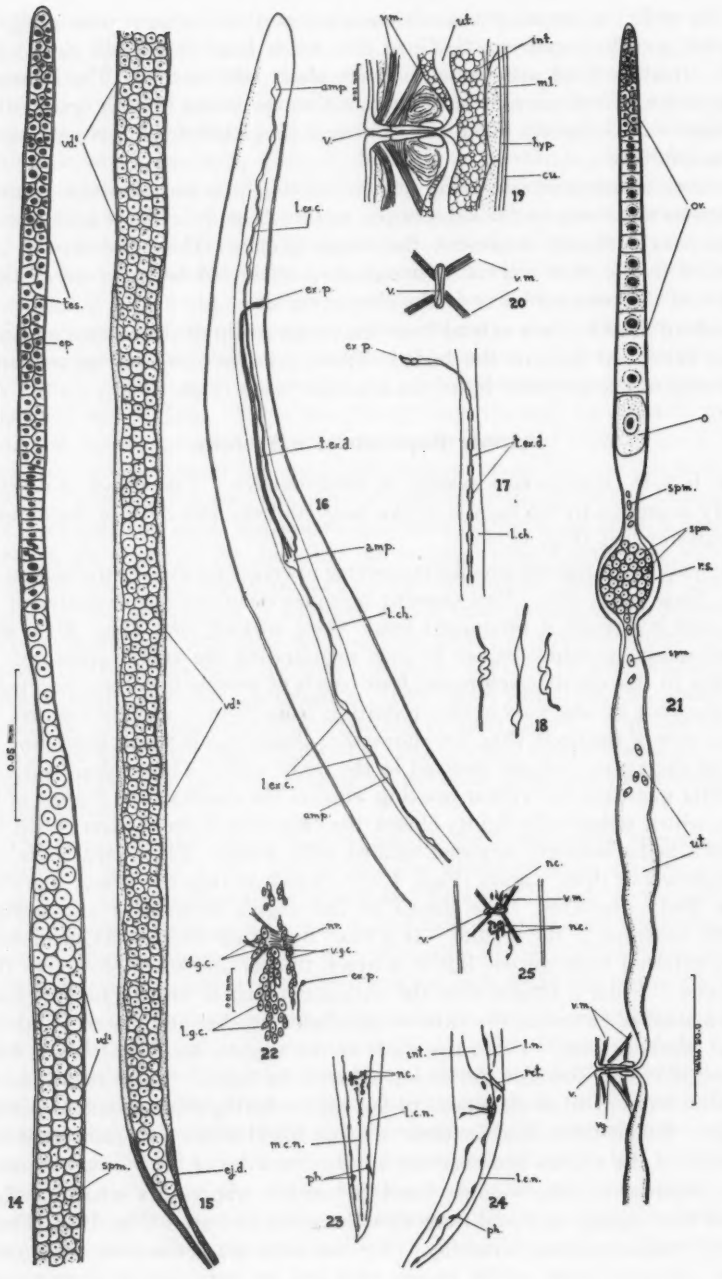
Bands of muscle fibers extend from the posterior lip of the rectum or cloaca to the subdorsal wall of the body. These muscles probably serve in the movement of the posterior lip of the anus or cloaca (Figs. 9, 10).

Female Reproductive System

The female reproductive system is amphidelphic. The vulva is situated slightly posterior to the middle of the body and the two ovaries are opposed and outstretched (Fig. 1).

The vulva is a narrow slit-like transverse aperture on the ventral side of the body (Figs. 1, 19, 20). This opening occupies one-third the diameter of the body and is devoid of prominent lips. On a surface view (Fig. 20) a well-formed muscular sphincter can be seen surrounding the vulvar aperture. In addition to this circular sphincter, four bands of muscle fibers are inserted at the two ends of the vulvar slit, radiating from it in a stellate fashion. A lateral view of the body (Fig. 19) shows that these muscle bands arise from the sides of the vulva and are inserted in the body wall. These, along with the sphincter guarding the vulvar opening, control the opening and closing of the vulva, which is normally tightly closed, the two sides of the transverse slit-like aperture being strongly apposed against each other. The vulva leads into the narrow but deep vagina (Figs. 1, 19), which in depth is about one-third of the body diameter. The lumen of the vagina is straight and narrow, slightly widening at the middle. It is lined by cuticle which is a continuation of the external body cuticle but it is much thicker and more refractive than the latter. From a lateral view the cuticular lining of the vagina appears to be composed of two rods, the anterior pair being smaller and placed in tandem to the posterior pair. From the walls of the vagina, bands of muscle fibers proceed obliquely towards the lateral sides of the body. These muscle bands probably serve to dilate the lumen of the vagina during copulation and passage of eggs. Beside these muscles there are four bladder-like cells associated with the walls of the vagina and situated on the two sides of the vaginal lumen.

The narrow slit-like vagina, lined by cuticle, represents what has been termed the 'vagina vera' and leads directly to the two uteri (Fig. 19). Thus a distinct 'vagina uterina' is lacking. The two uteri are at the inner termination of the cuticular lumen of the vagina vera and are very narrow at this point.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Both the uteri, however, widen immediately and run in opposite directions, lying parallel to the intestine. The intestine itself becomes narrow in diameter at the point of junction of the vagina and the uteri, evidently to make room for the deep vagina. In some specimens this region of the uteri was found to be swollen in two chambers and may probably serve as an ovejector although there is no demarcation whatsoever of a distinct structure.

The distal ends of the two uteri are modified to form two seminal receptacles (Figs. 1, 21) which are oval in shape and are demarcated from the ovaries and the uteri by a slight constriction on either side. These seminal receptacles are invariably found filled with sperms even in very young females. The seminal receptacles on each side of the uteri lead into the ovaries. There does not seem to be any particular part of the reproductive system which might be termed an oviduct.

The ovaries (Figs. 1, 21) are long tubular sacs lying straight and parallel to the intestine. The walls consist of a thin epithelium. There is no central rachis. At the blind end of each ovary there are two–three oöcytes arranged in a single row after which the oöcytes are packed in two irregularly arranged rows for a short distance. Posteriorly these reproductive elements again become arranged in a single row, ultimately giving rise to large ova which fill the posterior region of an ovary. Although all the females have mature ovaries with fully formed ova and seminal receptacle full of sperms, I was not able to observe a single female with eggs in the uteri. The uteri in most cases were found to contain a few sperms which also seem to migrate up into the ovary from the seminal receptacles (Fig. 21).

Male Reproductive System

The single testis (Figs. 2, 14, 32) consists of a finger-like core of tissue extending 0.81–1.10 mm. from the anterior end of the body. It is covered over by a thin epithelium which is continuous with the epithelium of the gonaduct. There is no central rachis and the spermatocytes lie closely packed in the testis. At the tip there is a single cell behind which the spermatocytes are arranged in two irregular rows. As the testis proceeds posteriorly the reproductive elements are arranged more regularly in two rows. Still further back they become arranged into a single row and also become larger in size. At the extreme posterior end of the testis, spermatozoa are formed. These are large rounded structures with a central nucleus. Silver impregnation stains the nuclei of all the germinal elements of the testis and it is difficult to determine the various zones of development along the length of the testis.

FIGS. 14–25. *Radopholus gracilis*. FIG. 14. Anterior region of the male reproductive system. FIG. 15. Posterior region of the male reproductive system. FIG. 16. Excretory system of the female. FIG. 17. Terminal excretory duct. FIG. 18. Ampullae of the posterior canal. FIG. 19. Vulvar region of female. FIG. 20. Vulva in surface view. FIG. 21. Female reproductive system (only one half shown). FIG. 22. Nerve ring and associated nerve cells. FIG. 23. Nerve supply of the phasmids in female tail. FIG. 24. Same on a lateral view. FIG. 25. Nerve cell groups in the vulvar region.

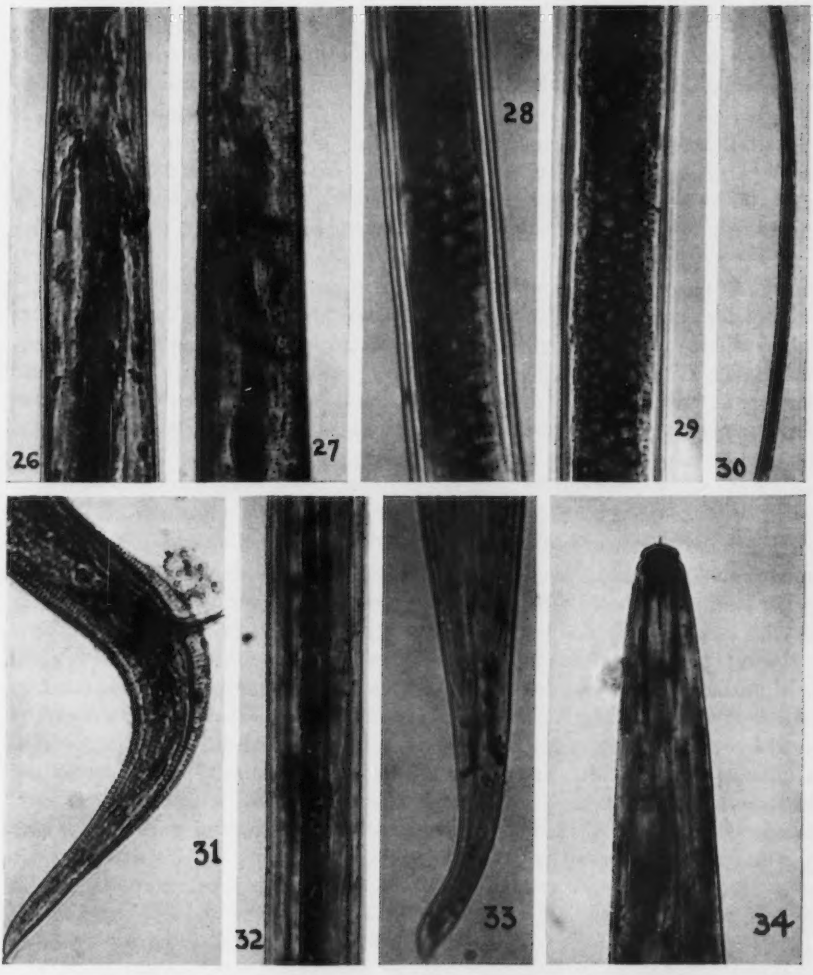
The testis passes imperceptibly into the next part of the male gonaduct, the vas deferens (Figs. 14, 15). The epithelial covering of the vas deferens is continuous with that of the testis. The anterior part of the vas deferens is narrow but as it proceeds caudad it widens out till it occupies the major part of the interior of the body, the intestine becoming very narrow in this region. The vas deferens is divisible into three regions which are not demarcated from each other superficially but are morphologically different. The anterior region of the vas deferens is tubular and thin-walled (Figs. 14, 15, 28). The region following it has thick glandular walls (Figs. 15, 29) which appear to be composed of glandular epithelium. The posterior region of the vas deferens narrows posteriorly and has thick muscular walls (Fig. 15). The lumen in this region also becomes very narrow. This part probably corresponds to the ejaculatory duct of other nematodes and opens into the cloaca from the ventral side. The actual relation of the intestine, ejaculatory duct, and the cloaca could not be made out clearly.

The two spicules are equal in size and symmetrically placed (Figs. 2, 11, 13). Each spicule has a slightly expanded head and a fine membrane along the concave border, which extends from the tip to two-thirds of the spicule length. The tips of the two spicules are bluntly pointed. Both the spicules appear to be grooved along their length. From the head of each spicule short but stout bands of muscle fibers extend to the lateral sides of the body wall (Fig. 11). These are probably the retractor muscles. Protractor muscles of the spicules could not be seen. The gubernaculum is plough-shaped with only the dorsal border showing heavy cuticularization. It is lodged on the dorsal wall of the cloaca. Short muscular bands are inserted between the gubernaculum and the dorsal wall of the body (Fig. 11).

Nervous System

The following observations on the anatomy of the nervous system, although far from complete, may serve as a basis for further study.

The central nervous system consists of a nerve ring surrounding the oesophagus about midway between the oesophageal bulb and the beginning of the intestine. This nerve ring is inclined at an angle with the oesophagus, the forward tilt being on the dorsal side of the body. The nerve ring seems to be mainly of a fibrous nature as no nuclei could be seen within the ring proper. Associated with the nerve ring are a large number of cells which have granular cytoplasm and deeply staining nuclei. These cells in most cases are surrounded by a clear mass of tissue. The nuclei of the various cells stain differently. The cells are all closely grouped together to form the various ganglia associated with the nerve ring. Since I was not able to delimit with exactitude a particular cell group from an adjoining one, owing to the fact that the component cells are visible in different focal planes, and since the nature of the different component cells is not clearly understood, I have not assigned the different ganglionic names to these cell groups.



FIGS. 26–34. *Radopholus gracilis*. Photomicrographs. FIG. 26. Nerve cells associated with the nerve ring on a lateral view. FIG. 27. Same on a ventral view. FIG. 28. Anterior region of the vas deferens with thin walls. FIG. 29. Posterior region of the vas deferens with glandular walls. FIG. 30. Male reproductive system. FIG. 31. Caudal region of the male showing striations on the caudal alae. FIG. 32. Anterior blind extremity of the testis. FIG. 33. Phasmids on the female tail. FIG. 34. Anterior region of the female showing amphids. All specimens silver impregnated.

From a lateral view of the body, with the vulva and excretory pore lying to the left side, a group of 27 cells could be seen which are closely packed together (Figs. 22, 26). These cells lie for the most part posterior to the nerve ring, overlying the beginning of the intestine.

From the dorsal side of the body a group of seven cells was observed lying almost at the level of the nerve ring. One of these cells is much larger in size than the adjoining cells.

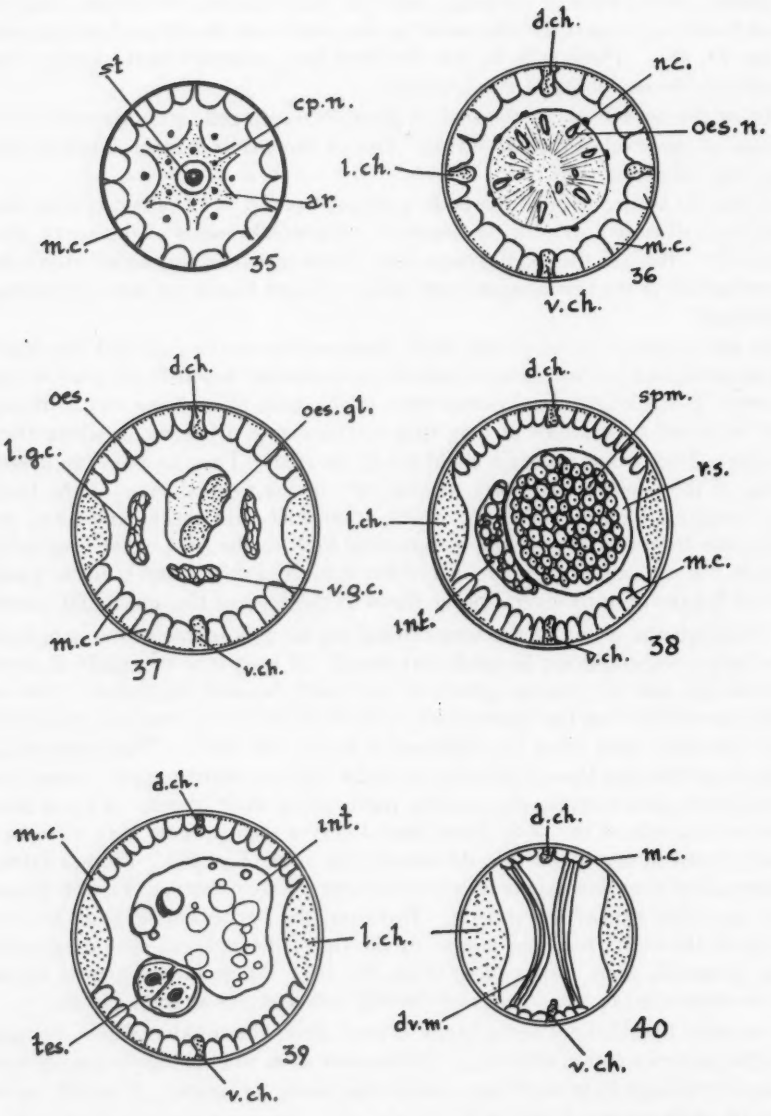
From the ventral side of the body a diffused group of 13 cells could be seen which are disposed on both the anterior and posterior sides of the nerve ring (Fig. 27). Besides these cell groups, a group of eight cells is situated above the anterior half of the oesophagus from which fibrous bands are seen proceeding cephalad.

In the anterior region of the body, between the nerve ring and the head, six longitudinal nerves are seen proceeding anteriorly towards the head of the worm. These longitudinal nerves were visible only along their middle region and have the appearance of long thin spindles with a few nuclei along their course. Their point of origin could not be located. They probably represent some of the cephalic papillary nerves. From the ventral view of the body two amphidial nerves (Fig. 3), which stain well with methylene blue, are traceable from the level of the oesophageal bulb to the base of the amphidial pouch. Each amphidial nerve is a rather stout and thick band of nerve tissue which breaks up into several nerve fibers at the base of the amphidial pouch.

Although the origin of the longitudinal nerves proceeding posteriorly from the nerve ring could not be made out clearly, at least four longitudinal nerve trunks are seen at various points of the body beyond the vulva. Two of these are situated on the lateral sides of the body, one runs along the middorsal and the other runs along the midventral line of the body. The longitudinal trunks on the two lateral sides of the body follow a slightly wavy course and show nerve cells with deeply staining nuclei along their length. Viewed from the ventral side of the body these lateral nerves seem to pass very close to a large group of nerve cells placed around the anus (Fig. 23). From a lateral view each of these lateral nerves seems to enter a large nerve cell (Fig. 24) placed one on either side of the rectum. However, the nerves continue on the two sides of the body, reaching almost to the tip of the tail and also innervating the phasmids along their course (Figs. 23, 24). These lateral nerves appear to be connected to the dorsal and ventral nerve trunks at some places.

A single longitudinal nerve trunk is seen along the middorsal line, running to the posterior end of the body. It does not show thickenings of a ganglionic nature although light swellings can be seen along its course. It could not be traced at its posterior extremity.

Along the midventral line of the body a longitudinal nerve trunk can be traced at a few places, particularly behind the vulvar region. This nerve trunk shows a large number of nuclei along its course. Its course at the posterior extremity, however, was not clearly seen. On the anterior and posterior sides of the vulva a group of cells is seen, the anterior group consisting



FIGS. 35–40. *Radopholus gracilis*. Semidiagrammatic cross sections of the body. FIG. 35. Cross section behind the head of female showing the arcade. FIG. 36. Cross section through the oesophageal bulb region. FIG. 37. Cross section below the level of the nerve ring. FIG. 38. Cross section at the level of receptaculum seminis. FIG. 39. Cross section at the level of the blind end of the testis. FIG. 40. Cross section through the postanal region of the female.

of eight cells and the posterior group consisting of nine cells (Fig. 25). The nuclei of these cells stain deeply. Similarly a group of cells disposed in a circle is also seen at the vicinity of the anal aperture (Fig. 23).

The phasmids (Figs. 12, 23, 24, 33) are located on the two lateral sides of the body in the tail region of both sexes. Each phasmid consists of a globular mass of cells from which a narrow duct leads to the exterior, opening at a minute pore situated on the lateral field of the cuticle. The two phasmids are generally placed asymmetrically, one being markedly more anterior in position than the other. However, their position varies in different specimens of both sexes and they may lie exactly opposite to each other. They are innervated from the lateral nerves (Figs. 23, 24).

The amphidial pores are situated on the two lateral lips. They lead into two short ducts, which swell out into small pouches at their posterior region to form the amphidial pouches (Figs. 3, 34). The nerve supplying the amphids breaks up into several small nerve fibers at the base of the amphidial pouch.

The 'hemizonid', described by Goodey (4) in tylenchids, is also present in this species. It is represented by a lens-like structure beneath the cuticle slightly anterior to the excretory pore. It is very easily distinguished from the surrounding tissue because of its highly refractive nature.

Habits and Habitat

These nematodes were found living inside the older and thicker roots, lying imbedded between the epidermis and the cortex. While in this position the piston-like movements of the stylet of individuals have been observed, indicating that the nematodes were feeding on the tissues or juices of the roots. The roots, however, were not the healthiest ones and were partly decayed. On the other hand, no other species of nematode was found inside the tissue of the root except *R. gracilis*, although several other species of nematodes were collected from soil around the roots. This species seems to be perfectly adapted to an aquatic environment, as the roots of wild rice were collected from beneath the surface of water. Further, the nematode does not seem to be affected by low temperatures, as the first sample of roots was collected from water beneath a frozen riverbed (approximate temperature of water, 4° C.) after cutting through 1 ft. thickness of solid ice, and the worms were recovered from these roots were living normally. The species also seems to be able to thrive well in water with very poor oxygen content, as the roots which were stored in water full of rotting vegetation for months together yielded worms which were as healthy and normal as those collected from fresh roots.

Acknowledgments

I acknowledge with thanks the award of a Postdoctorate Fellowship by the National Research Council of Canada in collaboration with the Department of Agriculture, that enabled me to undertake the present studies. I am deeply indebted to Dr. A. D. Baker, Officer-in-Charge, Nematology Section,

Science Service Laboratory, Ottawa, for going through the manuscript and for the encouragement, valuable suggestions, and criticism received throughout the course of the work, as well as for the free access to his personal library.

Abbreviations Used in Figures

amp.—amphid; amp.n.—amphidial nerve; an.—anus; ar.—arcade; c.a.—caudal alae; c.f.—cuticular framework; cp.n.—cephalic papillae nerves; cu.—cuticle; d.—duct of the phasmid; d.g.c.—dorsal ganglion cells; d.o.—dorsal oesophageal gland; ep.—epithelial lining; ej.d.—ejaculatory duct; ex.p.—excretory pore; gb.—gubernaculum; hyp.—hypodermis; il.—intestinal lumen; inc.—incisures; int.—intestine; ip.—inner circlet of papillae; l.ch.—lateral chord; l.c.n.—lateral caudal nerve; l.ex.c.—lateral excretory canal; l.g.c.—lateral ganglion cells; l.l.—lateral lips; l.n.—lateral nerve; m.—stylet muscles; m.c.—muscle cells; m.c.n.—median caudal nerve; ml.—muscular layer; nc.—nerve cells; nr.—nerve ring; o.—ovum; o.b.—oesophageal bulb; o.l.—oesophageal lumen; o.n.—oesophageal gland nucleus; oes.—oesophagus; oes.gl.—oesophageal gland; oes.n.—oesophageal nuclei; op.—outer circlet of papillae; ov.—ovary; p.—phasmidial pore; ph.—phasmid; r.—cuticular ridge; r.gl.—rectal glands; r.m.—rectal muscles; r.s.—receptaculum seminis; rct.—rectum; sv.o.—subventral oesophageal gland; sm.l.—submedian lips; sp.—spicules; sp.m.—spicular muscles; spm.—sperms; st.—stylet; t.—tail; t.ex.d.—terminal excretory duct; t.str.—transverse striations; tes.—testis; tp.—terminal process; ut.—uterus; v.—vulva; v.ch.—ventral chord; v.g.c.—ventral ganglion cells; v.m.—vulvar muscles; vd.[1]—thin walled region of the vas deferens; vd.[2]—glandular walled region of the vas deferens; vg.—vagina; vl.—oesophageal valve.

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THE BIOLOGY AND PATHOLOGY OF THE TAPEWORM TAENIA HYDATIGENA IN DOMESTIC AND WILD HOSTS[1]

G. K. SWEATMAN[2] AND P. J. G. PLUMMER[3]

Abstract

Hepatic fibrotic foci indicative of embryonic cysticerci first appeared in lambs with 7-day infections, and macroscopic cysticerci (1 to 9 mm.), most within haemorrhagic streaks, occurred at 10 days. Migration of cysticerci into the abdominal cavity from streaks and open pits in the liver capsule occurred in 18- to 25-day infections, and from the omentum in 25- and 30-day infections. Cysticerci in the abdominal fluid were last observed at 43 days. Most cysticerci that remained within the liver parenchyma of lambs became entombed within caseous and fibrotic matter, and failed to complete their rostellar development irrespective of the period of infection. Those at other sites developed suckers and complete hooks between 34 and 53 days. Cysticerci within the parenchyma of a moose completed their development. Hyalinosis of some omental cysts in lambs occurred at 25 days, but the cysticerci within remained viable until at least 53 days when some were infiltrated with caseous matter. A fibroma was associated with an omental cyst. Crystallized flakes occurred in teased hepatic haemorrhagic streaks and omental cysts in 4-month infections. Cysts displayed subsequent size regression. Adhesions and ribbons of fibrotic tissue, most involving the liver and diaphragm or the omentum and body wall, occurred initially in infections of 30 and 61 days respectively.

In infections longer than 53 days, the number of viable or degenerate cysticerci in lambs and swine at sites exclusive of the liver parenchyma was proportional to the total number of cysts. The increment in the size of these viable cysticerci increased at the same rate both before and after complete rostellar development. Only cysticerci with complete rostellar hooks infected dogs. Prepatent periods in dogs spanned about 2 months, and strobilar longevity varied from 4½ to at least 11½ months.

Introduction

Küchenmeister established the relation between taeniid tapeworms and cysticerci in 1852. Ten years later, Baillet (1) published his experiments on *Taenia hydatigena* Pallas 1766, demonstrating the life cycle and migration of the cysticerci from the liver into the abdominal cavity. Leuckart, according to Bronn (4), and Railliet (19) ascertained the period of infection required for the occurrence of cysticerci and haemorrhagic streaks in the liver, but only desultory data are available on the subsequent life of the tapeworm.

The pathologic condition of livers infected with *T. hydatigena* is called hepatitis cysticercosa. Most natural infections, however, involve few cysticerci and only cause mild local damage, but some have produced extensive haemorrhage and peritonitis that contributed to the death of the host (2, 8, 17). Death of a lamb was reported also by Dromey and Campbell (10) following ligation of the gut by a cysticercus. A cysticercus attached to the urinary bladder of a domestic goat caused spasmodic and apparently

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Contribution from the Animal Pathology Division, Canada Department of Agriculture, in co-operation with the Institute of Parasitology, Macdonald College P. O., Que., Canada.
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painful micturition (3). Graham and Gordon (13) noted photosensitization in a Merino wether associated with a degenerate cysticercus occluding the bile duct. Condemnation of lamb livers at abattoirs stimulated the conduction of the experimental infections of Whitten and Batham (24) and Pullin (18), who observed liver damage caused by the parasite. These reports, together with others on the incidence of the parasite in wild and domestic animals, indicate that *T. hydatigena* is encountered frequently throughout the world, but, even so, remarkably little has been written on its biology. The present study includes various biological observations on the tapeworm in definitive and intermediate hosts, with the primary objective of correlating the development of the cysticerci with the corresponding pathology after known periods of infection.

Materials and Methods

Ova were teased from gravid proglottids, passed through fine mesh bolting silk, and stored in physiological saline in a refrigerator (34° to 40° F.). A total of 800 ova was counted as required, placed in a gelatin capsule with moistened feed, and fed to each experimental animal. Swine were of various ages, but each lamb (a purebred Shropshire) was exposed when 6 weeks old. To maintain a constant age, it was necessary to keep the ova between 1 week and 3 months to accommodate variations in the birth dates. Lambs were maintained with their ewes in a single flock using standard procedures of farming practiced in eastern Canada. This necessitated taking measures to prevent infection of other animals in the flock from capsules that might be coughed up onto the pasture, or by ingested ova that might pass unchanged through the digestive tracts of lambs. Therefore, following exposure, each lamb was maintained in a corral and harnessed with a faecal collecting bag. The bags were emptied daily for a week, and then the animal was returned to the flock.

Animals were autopsied with infections ranging between 6 days and 9 months. The gross pathology was observed, and the abdominal cavity rinsed with physiological saline to collect cysticerci from the abdominal cavity. Cysticerci in the omentum, mesenteries, etc., were frequently examined while still *in situ*, while those within the liver parenchyma were collected by cutting the organ into many small pieces and allowing them to soak overnight in saline. The length of each cysticercus, carried out in saline after cessation of muscular movements and before fixation, was measured from the invaginated neck to the opposite border following its removal from the host membranes. Specimens for histopathological study were collected soon after the death of the host, fixed in 10% formal saline, sectioned at 4 microns, and stained with haematoxylin–eosin.

Results

The degree of infectiousness and susceptibility of domestic animals to the parasite was demonstrated using material derived from both wild and domestic herbivores. Dogs excreted proglottids of *T. hydatigena* following

ingestion of cysticerci from naturally infected lambs or from moose (*Alces americana*) that had been collected far from any agricultural region. Ova from either source successfully infected each of 29 lambs and five hogs, with the possible exception of two lambs killed before the period necessary for the appearance of macroscopic lesions. Some of the above infections demonstrate that the tapeworm that cycles normally between wild canids and moose (and probably other cervids) is capable of transfer to domestic animals. The total number of cysticerci in lambs with infections of a duration of 10 to 270 days (9 months) varied between 18 and 132 with 55 as the average. This is a minimum value since it is unlikely that all cysticerci were extricated from the washed slices of liver parenchyma. Since each lamb had ingested 800 ova, the average percentage infectiousness of the worm based on the number of recovered cysticerci was only 7%.

Development of Cysticerci

The development of the cysticerci, together with the host's response to the infection, was followed in several animals infected experimentally. White fibrotic foci measuring 0.5 to 2.0 mm. occurred immediately below the liver capsule of two lambs killed 7 days following exposure, although one lamb killed after 6 days and another after 8 days showed no obvious signs of infection. Greater numbers of these fibrotic areas were present in lambs killed after 10 and 12 days. Localized inflammation occurred that was characterized microscopically by infiltration of neutrophiles. Similar lesions (Fig. 2) occurred in later infections when small macroscopic cysticerci (1 to 9 mm.) were present also at other locations. At 20 days, the former were characterized in the subcapsular tissue by fibrotic foci composed of proliferating fibroblasts and capillaries with a moderate infiltration of eosinophiles throughout the area. The capsule over the lesions was thickened and fibrotic.

Macroscopic cysticerci were first apparent in a 10-day infection. Infections in 10 lambs of 30 days duration or less contained cysticerci up to 10 mm. in diameter; but none contained rostellar hooks that were developed completely. Cysticerci in a 34-day infection contained both complete and incomplete hooks (generally the handles were undeveloped), the former occurring in 76% of the cysticerci from sites exclusive of the liver parenchyma.

TABLE I

SIZE OF CYSTICERCI AND THE PRESENCE OF COMPLETE ROSTELLAR HOOKS

Age of cysticerci (days)	Incomplete hooks		Complete hooks	
	Size (mm.)	No.	Size (mm.)	No.
34	5 to 11	19	9 to 18	60
34*	—	0	11 to 15	3
43	5 to 8	3	10 to 18	19
53	7 to 8	3	9 to 13	5

*Hog.

All these cysticerci measured 9 mm. or more, whereas those cysticerci with incomplete hooks generally measured less than 10 mm. Table I summarizes this, together with other infections up to 53 days' duration in which some cysticerci even after that period occurred without complete hooks, and measured less than 10 mm. All viable cysticerci exclusive of those within the liver parenchyma in infections of longer duration than 53 days measured 10 mm. or more and each possessed complete hooks. These results demonstrate that the presence of complete hooks in cysticerci of *T. hydatigena* depends on the size of the cysticercus (about 10 mm. or more), and is independent of the period of infection beyond the required minimum of 34 days.

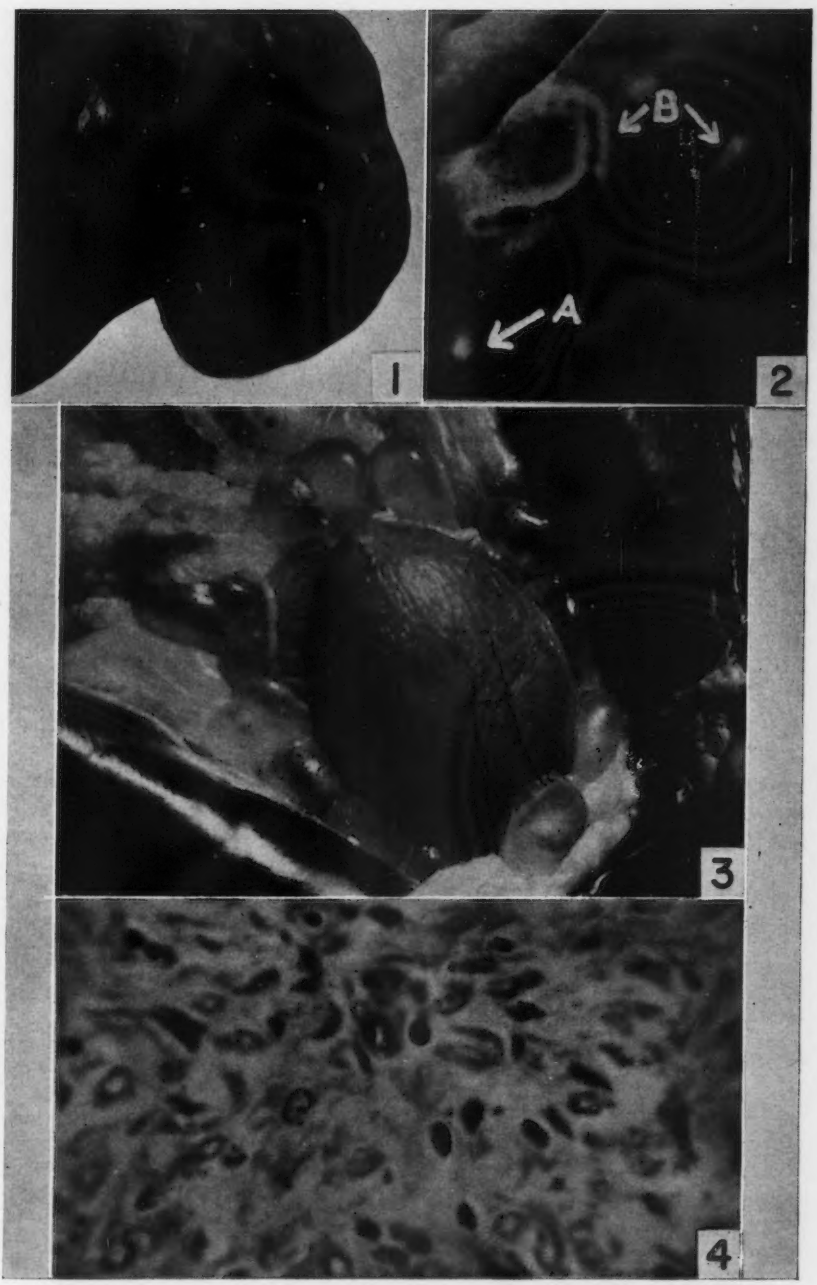
Cysticerci within the liver parenchyma accentuated the dependence of the rostellar hook development on the over-all size of the cysticercus. Let it be understood that the following remarks do *not* apply to those cysticerci immediately below Glisson's capsule that developed normally and were *on* rather than *in* the parenchyma. Up to 20 cysticerci were recovered from within the liver parenchyma of each experimental lamb with an infection of 10 days to 9 months. Except for three completely developed cysticerci in three different animals, all the remainder measured less than 10 mm. and were barren of hooks and suckers regardless of the period of infection. Observations in moose, however, showed complete development of two cysticerci within the liver parenchyma of one cow moose when undeveloped cysticerci were absent. Many undeveloped cysticerci in lambs were elongated and conformed to the shape of the surrounding haemorrhagic streak. As each streak underwent repair, the cysticerci became entombed within fibrotic and caseous matter. Hence, a large majority of cysticerci within the liver parenchyma of lambs maintained their viability for long periods but apparently never reached a suitable location to grow sufficiently to complete their rostellar development.

Hepatic Lesions and Migration of Cysticerci

The period of the infection during which cysticerci migrate from the liver and other tissues into the abdominal cavity as well as their state of development and associated lesions were observed in experimental lambs. Haemorrhagic streaks composed of blood coagula (Fig. 1) occurred throughout the liver parenchyma in infections of 10 to 20 days' duration. Similar lesions were observed in naturally infected moose and deer (*Odocoileus* sp.) from northern Canada in November. Streaks in experimental lambs were of various lengths up to 5 cm., which, microscopically, represented destroyed parenchyma and many degenerate erythrocytes associated with abundant hematogenous

FIG. 1. Haemorrhagic streaks, each containing one or two cysticerci, in a 10-day infection.
FIG. 2. Typical liver lesions in a 20-day infection.
A. Fibrotic focus with embryonic cysticercus.
B. Haemorrhagic streak repaired peripherally.
FIG. 3. Cysts located in the vicinity of the urinary bladder.
FIG. 4. Fibroma associated with an omental cysticercus in a 30-day infection.

PLATE I



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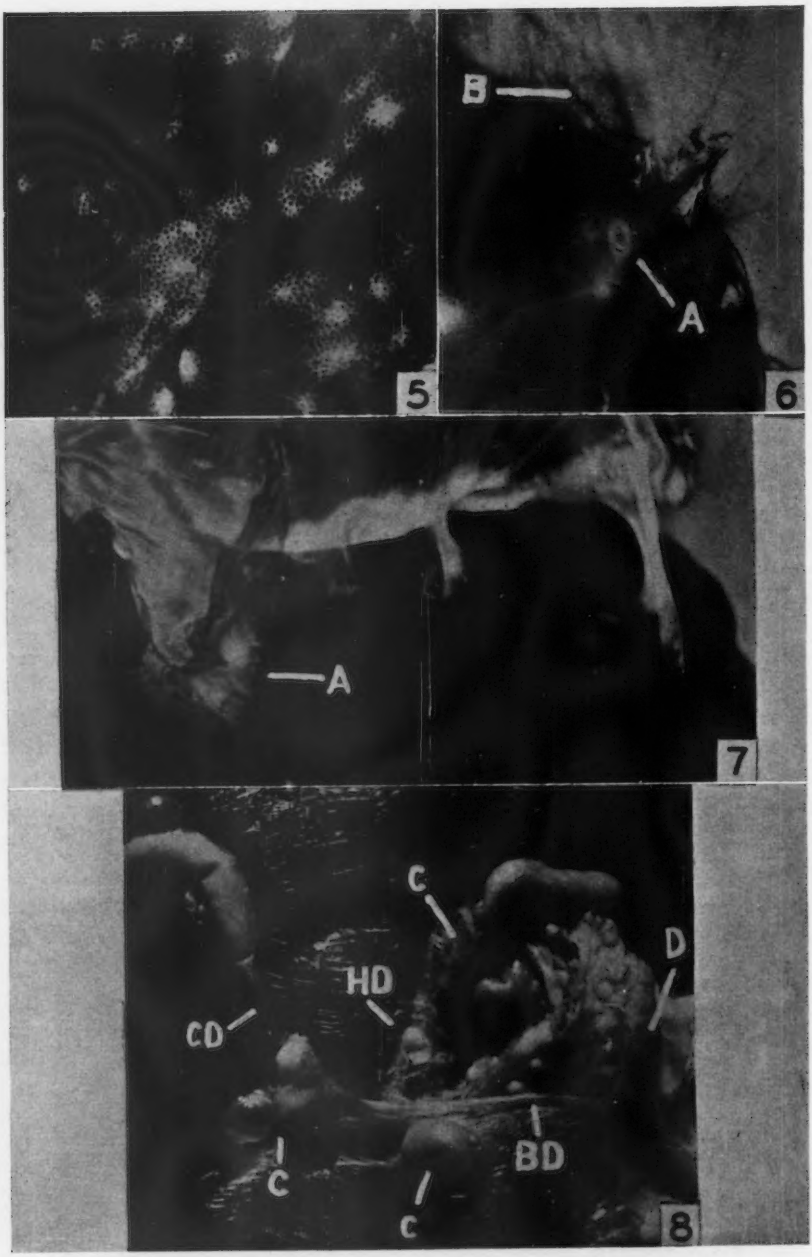

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pigment, some of which had undergone phagocytosis. The area was surrounded by a broad zone of cellular infiltration and proliferation composed of numerous eosinophiles and fewer lymphocytes and plasma cells. Some fibroblastic proliferation and capillary budding occurred also, suggesting a rapid repair process. A similar tissue reaction, with the addition of proliferating bile ducts, occurred in many periportal areas. Some streaks during this 10- to 20-day period were repaired peripherally as in Fig. 2. This more advanced stage of scarring was indicative of less haemorrhage and greater fibroblastic proliferation than in the streaks of Fig. 1. Infections of 25 days and longer contained yellow or white caseation throughout many streaks, especially those located within the parenchyma. The former color may have indicated the presence of bile. Replacement of obvious blood coagula occurred in all streaks after 34 days. Similar repaired streaks in a 43-day infection displayed periportal lesions suggestive of early cirrhosis. There were foci of necrotic parenchyma surrounded by epithelioid cells and giant cells of Langhans' type that were interpreted as being granulomatous in nature. Extensive fibroblastic proliferation had developed in the lesions, lymphocytes were abundant, and eosinophiles were present in fewer numbers than in infections of shorter duration.

Haemorrhagic streaks contained small cysticerci. Usually one, and never more than two, cysticerci occurred in each recent streak in lambs examined 10 and 12 days following exposure. Four streaks in a 12-day infection extended through the parenchyma and ended at the surface of the liver, where each contained a small cysticercus (1 to 4 mm.) located immediately below the liver capsule. These cysticerci had not ruptured the capsule in this animal, and none was observed in the abdominal fluid. Twenty cysticerci were recovered from the liver parenchyma following slicing and washing, and all moved actively in physiological saline at 37° C., demonstrating their viability. It was in an 18-day infection, however, that definite evidence occurred in the liver indicative of one source of cysticerci in the abdominal fluid. At that time, ruptures at the ends of some haemorrhagic streaks demonstrated former loci of the cysticerci. Similar observations were made in a 20-day infection with the addition of three pits in the surface of the liver that had haemorrhaged locally, but did not extend into haemorrhagic streaks. A small cysticercus projected from one of these pits through a rupture in the liver capsule. The last evidence of migration from the liver was a single

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FIG. 5. Fibrotic foci associated with small cysticerci in a pig's liver.
FIG. 6. Fibrotic film over parietal surface of liver.
A. Cysticercus in depression of, but external to, Glisson's capsule.
B. Thickened area of diaphragm.
FIG. 7. Adhesion (A) and ribbons of fibrotic tissue between liver and diaphragm.
FIG. 8. Occlusion of cystic duct by an encapsulated cyst.
C.D. Occluded cystic duct.
H.D. Enlarged common hepatic duct.
B.D. Enlarged common bile duct.
D. Duodenum.
C. Cyst.

puncture pit in a 25-day infection. Streaks, it will be recalled, were filled with caseous matter at this time. These results showed that, in infections of 18 to 25 days' duration, cysticerci migrated from the liver into the abdominal cavity from within the parenchyma by sinuous haemorrhagic streaks, or simply through Glisson's capsule when located at the liver's surface.

Some cysticerci located *immediately below* Glisson's capsule developed completely and remained viable throughout the 9 month period of study. Hyalinosis of the walls of other hepatic cysts* in a similar location occurred in infections of 61 days and longer resulting in an opacity and increased wall thickness up to 3 mm. At this time, some had become infiltrated with caseous matter and in certain instances the cysticercus was partially degenerate. Crystallized flakes were observed microscopically in hepatic streaks and adventitious layers of some *teased* cysts in infections of 120 days (4 months) or more, suggesting an advanced host reaction at that time.

It was established that most cysticerci must migrate from within the liver parenchyma or fail to develop completely. The necessity for this migration is realized if only completely developed cysticerci infect carnivores. Hence, large numbers of viable cysticerci with only partially developed scoleces and measuring 6 to 9 mm. were fed to two dogs which were negative at autopsy 3 weeks later. This suggests that cysticerci are not infective until hooks and suckers develop. Consequently, most hepatic cysticerci are not infective until they migrate to the surface of the liver or into the abdominal cavity.

Cysticerci in the omentum eventually developed complete scoleces, but even so, some undeveloped cysticerci migrated into the abdominal cavity. Ruptured encasements in the omentum from which cysticerci had presumably migrated, and some cysticerci projecting partly through the adventitious layers, were observed in 25- and 30-day infections. This period of migration was a little later than that noted from the liver.

Cysticerci in the Abdominal Fluid

The destination of cysticerci in the abdominal fluid was inferred from chronological infections. A single cysticercus of unknown origin occurred in the abdominal fluid in a 10-day infection. Most, however, were present in 18- and 20-day infections when 33 (69% of total number) and 26 (79%) cysticerci occurred respectively. Five lambs examined between 22 and 43 days after exposure contained 6 to 25 (22 to 30%) cysticerci in the abdominal fluid, and none occurred in 16 lambs killed after that period.

Cysticerci occurred in the ligaments joining the biliary ducts and liver in early infections, but mesenteric cysticerci were first observed in a 34-day infection and thereafter in 11 of 15 lambs until the last animal was examined after 9 months. It was also in infections of 34 days and longer that cysticerci occurred at diversified locations in the abdomen, including the peritoneum, renal capsule, gall bladder, diaphragm, rumen, spleen, ovary, uterus, and urinary bladder; an example of the last is shown in Fig. 3.

*'Cyst' is used throughout to include the parasite *and* the surrounding adventitious layers. Cysticercus is reserved for the tapeworm per se.

Pulmonary infections occurred also in 20- and 43-day infections. These, and probably some of the infections above, became established directly from the circulatory systems, but the presence of so many cysticerci at such varied locations in only later infections suggests that at least some sites may have been infected secondarily with cysticerci that were once loose in the abdominal fluid.

Omental Cysticerci and Lesions

Sequential changes were examined in omental cysts. Viable omental cysticerci were surrounded by two translucent hyaline membranes presumably of host origin. The necks of cysticerci were generally replete with calcareous granules, whereas their bladders contained a transparent fluid. Cysts of this kind occurred in most lambs with infections up to 9 months' duration, but a thickened, white, opaque encasement around the parasite was apparent in a few instances in a 25-day infection and some occurred thereafter in most lambs. One omental cysticercus in a 30-day infection was surrounded by a dense mass of cellular fibrotic tissue with extensive vascularization and little collagen indicative of a fibroma (Fig. 4). Opaque encasements contained viable cysticerci in early infections, and it was not before a 53-day infection that cysticerci were first ruptured and the cyst infiltrated with caseous matter. These cysticerci had ruptured bladders, were white and opaque instead of translucent, and generally had evaginated necks and scoleces. They were non-motile in physiological saline at 37° C. These, and not simply opacity of the cyst wall, were accepted as criteria of death. Disintegration of cysticerci was accompanied by a diminution in the size of the cyst, and an increase of consistency within. Cysts with degenerate cysticerci were identified by the presence of scattered rostellar hooks within the caseous debris. Such cysts, which measured between 2 and 12 mm. (average 5 mm.) in infections of 5 to 9 months, sometimes appeared as hard yellow bodies in the omentum, and, since fully developed rostellar hooks were present, the cysticercus must have regressed from a size of 10 mm. or more. Degeneration was sometimes complicated by the cyst becoming enveloped in omental fat. Serial sections of some cysts that had regressed in size showed a thick wall of connective tissue arranged in a circular pattern with scattered necrotic areas probably representing the dead cysticercus. The peripheral tissue of some cysts in an 82-day infection consisted of epitheloid cells and giant cells of Langhans' type making up a loose areolar matrix, and was indicative of a granulomatous reaction. A similar chronic lesion was noted previously in conjunction with hepatic haemorrhagic streaks in a 43-day infection. At 82 days, the centers of some omental cysts showed some calcification in section; and crystallized flakes, sometimes accompanied by larger crystallized lumps, were present in some teased degenerate omental cysts in lambs with infections of 5 months or more. This was a month after similar structures were observed in hepatic lesions. Similar crystallization occurred before that time in teased adventitious layers surrounding two *viable* cysticerci in a hog with a 92-day infection. Cysts with degenerate parasites occurring in swine at this time, like those

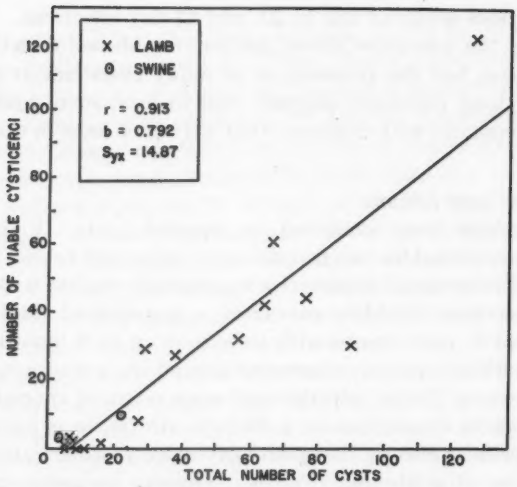

FIG. 9. Ratio of viable cysticerci to the total number of cysts.

in lambs, consisted of connective tissue growing in a circular manner. Changes in both hosts consisted therefore of opacity and thickening of the cyst wall, death of the cysticercus with caseous infiltration, calcareous degeneration, and size regression of the cyst.

Degree of Viability and Growth of Cysticerci

The ratio of degenerate to viable cysticerci from all 16 lambs and two hogs with infections of 53 days (when degeneration appeared initially) to 9 months was determined for cysts at all sites exclusive of the liver parenchyma. Cysticerci from the latter were excluded because of the uncertainty of recovering all of them. No correlation existed between the percentage of degenerate cysticerci in the animals and the period of infection during the time of these observations. Fig. 9 shows, however, that a positive correlation ($r = 0.913$; $d = 0.768$) did exist between the number of viable cysticerci and the total number of cysts during the period. The regression coefficient was highly significant ($t = 8.94$; $p < 0.001$), and the standard error of estimate (S_{yx}) was 14.87. This correlation shows that the number of viable or degenerate cysticerci in the animals was proportional to the total number of cysts infecting the animals; and the value of the regression coefficient ($b = 0.792$) indicates a 79% incidence of viable cysticerci. Since a more or less constant proportion of cysticerci died in all animals, then, insofar as the effect on mortality was concerned, the environment must have been similar in all animals.

The growth rate of cysticerci was inferred by measuring individuals of known age. Cysticerci from the liver parenchyma and any undergoing obvious degeneration at other sites were excluded from analysis, but all other cysticerci are included. The linear dimension of 587 living cysticerci

previously extricated from the host membranes was determined in physiological saline from the edge of the cysticercus at the invaginated neck supporting the scolex to the opposite side. Measurements were made when the cysticercus was momentarily quiescent to reduce the effects of rhythmic contractions and expansions.

Cysticerci with incomplete rostellar hooks may grow more rapidly than those with complete hooks. To determine this, observations from both groups were analyzed independently. Dispersal of points and spread of the 95% confidence bands in Figs. 10 and 11, as determined using the method

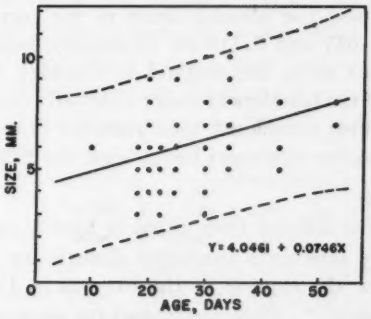

FIG. 10. Growth rate of cysticerci without complete rostellar hooks. (In some instances, a single point on the graph represents up to 20 individual observations.)

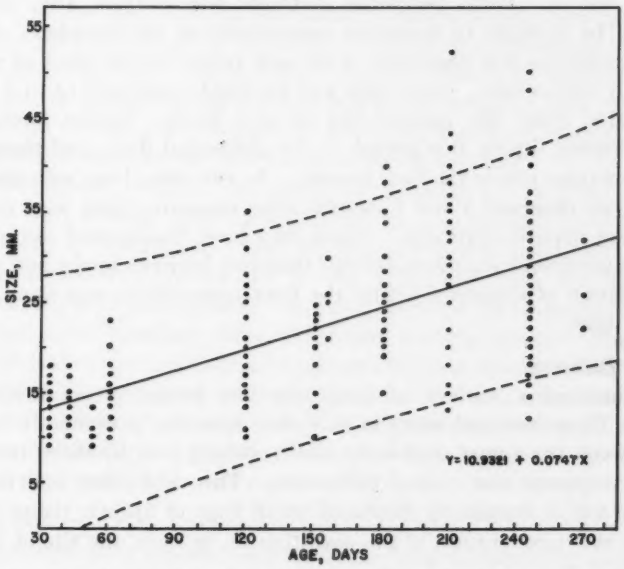

FIG. 11. Growth rate of cysticerci with complete rostellar hooks. (See Fig. 10.)

of Snedecor (21), show that the size of individuals at different ages varied between wide limits. The largest cysticercus measured 52 mm. and was observed at 210 days (7 months), even though observations were made 2 months beyond that period. Variations may be partly explicable by the elasticity of the bladder and diffusion of fluids through the membranes. The lines in both figures are parallel showing that the mean rate of increment in the size of cysticerci with incomplete hooks ($b = 0.0746$) was virtually the same as that of cysticerci with complete hooks ($b = 0.0747$). The lines are separated because of the larger size of the latter cysticerci. Both groups of data were highly significant (both $p < 0.001$) showing a definite relationship between size and time, and the fiducial limits of the regression coefficients at the 5% point were 0.031 and 0.118 for incomplete hooks and 0.068 and 0.082 for complete hooks using the method in Goulden (12). These data suggested that the size of the bladders of viable cysticerci continued to increase unabated even after having completed their rostellar development, and this condition continued in viable cysticerci for at least the first 9 months of life.

Hepatic Lesions in Swine

Hepatic lesions in swine differed from those in lambs and cervids. Three hogs with 12- to 34-day infections contained fibrotic foci with many short radiating branches below the capsule of the liver as in Fig. 5. Sometimes these are called "milk spots".* They contrasted the sinuous streaks observed in lambs with similar periods of infection. The Glisson's capsule is normally well developed in swine, but was even more thickened and fibrotic at the site of the lesions. Focal periportal cirrhosis was evident also, usually accompanied by a slight to moderate eosinophilia at the periphery of the scars. A cysticercus was associated with each teased lesion, most of which were partially degenerate. Only eight and six viable cysticerci (2 to 4 mm.) were extricated from the parenchyma of two livers. Unlike lambs, no cysticerci occurred during this period in the abdominal fluid, and there was no evidence of open pits in the liver capsule. In two other hogs with omental and other cysts observed 3 and 5 months after exposure, there were neither milk spots not hepatic cysticerci. These may have disappeared during this interval. Macroscopic reactions differed therefore between lambs and swine, and degeneration of cysticerci within the liver parenchyma was more rapid in the latter host.

Other Gross Pathology

Cysts induced a variety of local reactions in lambs not mentioned heretofore. Three omental cysts in a 30-day infection presumably caused friction between the rumen and body wall resulting in a localized inflamed area in the omentum and a local peritonitis. This, and other infections of 25 days and longer frequently displayed small flaps of fibrotic tissue (up to 10 mm.) on the outer surface of the omentum at, or near, the site of a cyst.

*Caused also by migrating *Ascaris* larvae, but this worm did not occur in these hogs.

Fig. 6 shows a thin fibrotic film over the parietal surface of the liver of a lamb with a 20-day infection, and corresponding to this was a thickened area of diaphragm. Within the region were two indentations in the surface of the liver in which cysticerci were lying external to the liver capsule. The abdominal fluid was cloudy. The cysticerci were doubtless from the abdominal fluid and became lodged between the liver and diaphragm causing the reaction.

Eight of 13 lambs examined with infections of 61 days or longer contained fibrotic ribbons or "tags" between the omentum and body wall, the liver and diaphragm (Fig. 7), or rarely the mesenteries and body wall. Up to 10 fibrotic ribbons occurred in one animal. They were generally about 1 cm. wide and 3 cm. long, although some were threadlike and others as long as 7 cm. Both viable and degenerate cysts were involved in ribbon formation. One cyst, or less frequently two or three cysts, occurred invariably at the omental, hepatic, or mesenteric end of the fibrotic ribbon rather than on the diaphragm or body wall.

Adhesions caused by cysts (Fig. 7) occurred initially in a 30-day infection, and thereafter in 8 of 16 lambs and two hogs. As many as four adhesions occurred in one animal; most of these involved the liver and diaphragm with some between the omentum and body wall, and one included the liver, diaphragm, rumen, and spleen. Like the fibrotic ribbons, one to three cysts occurred in the visceral organ involved in the adhesion.

Attachment of cysts to the ligaments joining the liver and biliary ducts is not uncommon. Fig. 8 shows an unusual situation in a 5-month infection where the largest (14 mm.) of two heavily encapsulated cysts pressed the cystic duct against the surface of the liver apparently preventing the flow of bile through that duct into the gall bladder. Bile was absent from the gall bladder, and both the hepatic and bile ducts were enlarged, presumably compensating for the lost function of the bladder and accommodating the flow of bile directly from the liver tissue to the duodenum.

Observations were made on two lambs, not included in any of the preceding data, that died mainly from haemonchosis 18 and 22 days after ingestion of 300 ova of *T. hydatigena*. One of the two lambs contained 33 small undeveloped cysticerci unattached in the abdominal fluid and 14 similar cysticerci in the liver parenchyma. One cysticercus in the omentum, however, was 10 mm. and possessed fully developed rostellar hooks. The second animal also had many undeveloped cysticerci but one that occurred immediately below the liver capsule was fully developed. It is unknown whether the lower initial dose of ova compared with the 800 ova given to each animal in the main experimental group, or sickness from the nematode, facilitated a more rapid development of some cysticerci.

Effect of Temperature on Cysticerci

The longevity of cysticerci removed from their surrounding membranes was determined in physiological saline at a few temperatures. Muscular

TABLE II

SURVIVAL OF CYSTICERCI AT DIFFERENT TEMPERATURES

Temperature (°C.)	Survival period
−21	45 to 60 min.
4 to 7	6 to 10+ days*
29 to 31	5 to 8 days
34 to 36	4 to 7 days

*See text for explanation.

contraction of the bladder or neck was the criterion of viability. Table II shows that freezing temperatures were rapidly fatal to cysticerci, while the longest survival was noted for cysticerci held a few degrees (4° to 7° C.) above freezing. The latter's survival period (6 to 10 days) was probably not a maximum value since it was necessary to warm these cysticerci periodically to induce muscular contraction. Three slightly motile cysticerci remained after 10 days and were fed to a dog. Another dog received a large number of non-motile cysticerci. Both dogs were negative at autopsy three weeks later; this fact suggests that non-motile and only slightly motile cysticerci are non-infective for carnivores.

Natural Infections of Cysticerci

Lambs from widely separated parts of Canada sent to Montreal abattoirs were infected occasionally with *T. hydatigena*. Eighteen omental cysticerci occurred in six of 185 lambs during one scrutiny, and on another occasion three mesenteric cysticerci infected one of 200 lambs. Infected swine and cattle occurred less frequently.

Sylvatic infections were observed also. Hepatic cysticerci, most of which occurred immediately below Glisson's capsule, numbered one to 20, and occurred in 28 moose (*A. americana*) from various places in Northern Ontario, together with a few cysticerci on the omentum, mesenteries, diaphragm, pancreas, and the ligament joining the bile duct and liver. The incidence was unknown, but appeared high. This was verified when infections were observed in 15 of 17 moose from Big Island, Lake of the Woods. All the adult moose ranged between one and 12+ years of age* and were positive; the uninfected animals being two of five calves examined, each of which was 8 or 9 months old. Infections occurred also in moose from northern Saskatchewan; deer (*Odocoileus* spp.) in Ontario, Quebec, Alberta, and British Columbia; barren-ground caribou (*Rangifer arcticus*) in the Northwest Territories; and reindeer (*Rangifer tarandus*) at Aklavik, N.W.T. These observations indicate that cysticerci may occur more frequently in moose and possibly other cervids than in domestic animals in Canada.

*Ages of moose were estimated according to available data on tooth wear by R. C. Passmore and A. T. Cringan, Ontario Department of Lands and Forests.

Infections in Definitive Hosts

The developmental rate and longevity were ascertained for adult *T. hydatigena*. Viable cysticerci from either moose or lambs infected five of seven dogs. In addition, each of 10 timber wolves (*Canis lupus*) from Northern Ontario was infected naturally with two to 11 worms that occurred in the anterior third of their small intestines.

Infected dogs excreted both gravid proglottids and independent ova in the faeces, the former occurring generally on the outside of the formed stool. Four dogs voided proglottids initially at 53, 54, 70, and 74 days following exposure, whereas the respective times for the excretion of eggs free of proglottids were 51, 53, 64, and 76 days, demonstrating that the latter were excreted generally before whole proglottids. Another experimental dog, autopsied 60 days following the feeding of four cysticerci, was infected with one worm composed entirely of immature proglottids. This, together with the data above, proves that prepatent periods are about 2 months and vary between wide limits.

Excreted proglottids varied between two extremes. Most were white, motile, and only partially, if at all, degenerate, but generally contained very few ova with fully developed membranes. Contrasting these were faecal-stained, partially degenerate proglottids containing numerous complete eggs. The estimated totals in two of the latter proglottids were 27×10^3 eggs and 63×10^2 eggs,* indicating a high egg production. Many excreted proglottids remained active for a day or so, and during that time frequently moved up to three feet from the deposited faeces by rhythmic undulatory movements.

Longevity of the strobilar phase of the parasite varied in different dogs. Five months (146 days) following exposure, a dog excreted 39 independent proglottids together with two unbroken sections consisting of 47 and 91 proglottids. The dog was then autopsied and found negative. One dog, $4\frac{1}{2}$ months (133 days) after ingesting cysticerci, excreted an entire worm consisting of 192 proglottids as well as the scolex, and the host was negative at autopsy.

An infection of five worms occurred in a dog, which accounts for all the cysticerci fed the animal, when autopsied $5\frac{1}{2}$ months (166 days) following ingestion. During this time, the dog had excreted a daily average of 3.7 proglottids (range 0 to 24), together with two unbroken sections of 73 and 117 gravid proglottids 152 and 164 days following exposure. Another dog was autopsied $11\frac{1}{2}$ months (342 days) following the ingestion of four cysticerci and found infected with a single worm. This dog had excreted proglottids regularly, except during the last three months when there were two periods of 3 weeks each during which proglottid excretion had ceased. The infections in dogs showed that proglottids were excreted regularly, and that the longevity of the strobilar worm varied from $4\frac{1}{2}$ to at least $11\frac{1}{2}$ months.

*Each was estimated from three dilution samples. Differences between counts were attributable to chance according to the chi-square test of Snedecor: $x^2 = \Sigma(X - \overline{X})^2/\overline{X}$ (both $p>0.80$).

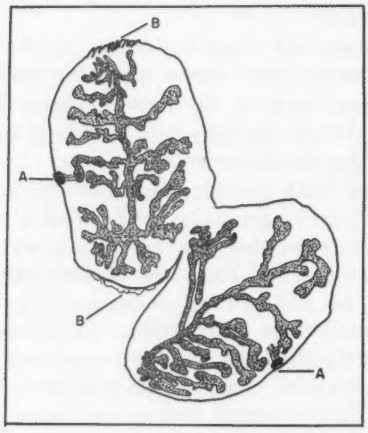

FIG. 12. Atypical proglottid of *T. hydatigena*.
A. Genital atrium.
B. Strobilar attachment.

Malformations occur rather frequently in cestodes. A recent summary was made by Burrows and Klink (5) with a new observation on *Taenia saginata*. In *T. hydatigena*, Canavan (6) has observed a worm with a scolex possessing six suckers and having two strobila fused for their entire length. Endogenous budding within a cysticercus was observed by Crusz (7). In the present study, an atypical proglottid was excreted by a dog 2 months following exposure. Fig. 12 shows a supernumerary proglottid fused with a normal one that had belonged to the main chain of the strobila. Two genital atria and associated canals were present, and a gravid uterus occurred in each proglottid with no indication of fusion between them. This suggested that their reproductive organs had functioned independently.

Discussion

Results suggest that timber wolves in Northern Ontario are infected commonly with *T. hydatigena*. This probably reflects the high incidence observed in moose and indicated in deer. Other infections were observed also from widely separated parts of Canada. At Aklavik, N.W.T., Whitten (23) observed one to 16 viable hepatic cysticerci in 14% of 575 reindeer, and Love (15) has observed up to 18% incidence in wapiti (*Cervus canadensis*) in Alberta. A noticeably lower incidence appears to exist in domestic animals in Canada, contrasting the large number of infected farm animals in Australasia (16, 20, 24) and Uruguay (2). Descazeaux (9) suggested that the cestode was imported into Chile from Australia and New Zealand. In Canada, sylvatic infections indicate that *T. hydatigena* is indigenous and distributed geographically throughout most of the country independently of the artificial effect of introduced reindeer and domestic animals. The parasite in domestic

stock probably remains essentially isolated from that in wild hosts, as with *Echinococcus granulosus* (22). But transfer to *T. hydatigena* is possible. This was shown when cysticerci from moose infected dogs that excreted ova which in turn infected lambs and swine. Hence, the increasing number of livestock in frontier settlements in close association with wild animals creates a potential means of transfer of this parasite to farm animals.

Leuckart (*in* Ref. 4) observed that cysticerci possess rostellar hooks and suckers 1 month after infection. This is essentially true, but applies only to some cysticerci; the necessary period reported herein in lambs and swine at sites exclusive of the liver parenchyma ranging between 34 and 53 days. Some cysticerci within the liver parenchyma of a moose completed their rostellar development, and Whitten (23) made similar observations in reindeer. Lambs and swine, unlike these cervids, generally contained undeveloped cysticerci (1 to 9 mm.) *within* the liver parenchyma in infections of a duration up to 9 months. These cysticerci had become entombed within scar tissue 25 days following exposure of the host. However, only those cysticerci with complete rostellar development infected carnivores. In winter in Northern Ontario, cysticerci in the omentum and at the surface of the liver of moose became quickly frozen and non-infective, while those within the parenchyma maintained their viability for at least 48 hours. Subzero temperatures (−6° F.) maintained in the laboratory were lethal to unprotected cysticerci within an hour, verifying the former observation. Since moose and other cervids are for the most part hunted by man and killed by predators in winter, those protected cysticerci within the liver parenchyma may be of some importance in the maintenance of the sylvatic parasite. Prepatent periods in dogs are about 2 months, ova are infective immediately following excretion, and some adult worms live at least 11½ months, demonstrating that carnivores that become infected in winter would disseminate ova during the following summer when herbivores would be more likely to acquire the infection.

Tumors, mainly benign, have been associated with a variety of parasitic infections. Papillomata are a frequent feature of schistosomiasis; malignant sarcoma occurs regularly with the strobilocercus of *T. taeniaeformis*, and multiple peritoneal sarcoma and mesothelioma were produced by Dunning and Curtis (11) following the intra-abdominal injection of rats with this parasite. A fibroma was associated with an omental cysticercus in the present study. This may have developed from a chronic inflammatory reaction or granuloma, which occurred with other omental and hepatic cysticerci. Similar conjunctival and cutaneous granulomata in horses have been associated with larvae of *Habronema* sp. and *Draschia* sp. (20), and with dead cysticerci of *T. solium* in swine (14). Giant cells of the foreign-body type occurred with *T. solium*, whereas those in the present study on *T. hydatigena* were Langhans' type of giant cell. On the whole, pathological changes caused by the parasite were of localized importance, but of little significance to the over-all health of the host.

Acknowledgments

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STUDIES ON THE EVOLUTION OF THE OPHRYOSCOLECIDAE (CILIATA: OLIGOTRICHA)

I. A NEW SPECIES OF ENTODINIUM WITH "CAUDATUM," "LOBOSO-SPINOSUM," AND "DUBARDI" FORMS, AND SOME EVOLUTIONARY TRENDS IN THE GENUS ENTODINIUM[1]

G. LUBINSKY

Abstract

A new species of *Entodinium*—*E. simulans*—with an intraspecific variability similar to that of *E. caudatum*, is described. A parallel variability in *E. rectangulatum* is reported. It is shown that sets of the forms of the "caudatum," "loboso-spinosum," and "dubardi" types are produced by each of these three species of *Entodinium*. It is demonstrated that within the "caudatum–laterale" group of *Entodinium* there exists an evolutionary trend to the transformation of the left lateral groove into a narrow furrow, situated between the nuclear apparatus and the contractile vacuole.

Introduction

The present paper originated as an attempt to overcome difficulties encountered by the author in identifying some forms of *Entodinium* with caudal spination similar to that of *E. caudatum* Stein and *E. loboso-spinosum* Dogiel. These forms, found in the rumen contents of domestic ruminants in the Western Punjab, were easily identifiable with the aid of Dogiel's key to the genus *Entodinium* (11). The outcome was, however, discouraging, as the author, strictly following this key, was compelled to identify three obviously different species of *Entodinium* as *E. caudatum* and three related forms with "loboso-spinosum" type of caudal spination as *E. loboso-spinosum*.

This result was not entirely unexpected. Dogiel's key (11) to the genus *Entodinium* is based whenever possible on the characters of the caudal spination and does not mention such important traits as the position of the contractile vacuole (which he believed to be the same in all species of *Entodinium*) and the shape and development of the left lateral groove. Bhatia (2), who introduced into Dogiel's key the "laterale" group of *Entodinium* of Kofoid and MacLennan, has only partially corrected this defect (but simultaneously introduced into the key numerous errors). The overestimation of the taxonomic value of the caudal spination in the systematics of the Ophryoscolecidae, and particularly in that of the genus *Entodinium*, is the result of a tradition, established by the founder of this systematics, F. Stein. *E. caudatum*, described by Stein in a paper read in 1858 and published in 1859 (25), is characterized by him exclusively on the basis of its caudal spination; so is *E. loboso-spinosum*, described by Dogiel in 1927 (11). The position of the contractile vacuole is not mentioned in the descriptions of these species.

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In 1888 Schuberg (24) redescribed *E. caudatum* and gave good drawings of it (24, Pl. 12, Figs. 7–8). The specimens depicted by him have a very long left lateral groove and a contractile vacuole situated near the ventral side of the body ventrally to this groove. In his figures the length of the dorsal spine is conspicuous and equal to the body length. A similar specimen of *E. caudatum* was depicted by Fiorentini (12, Pl. 4, Fig. 4). In 1927 Dogiel (11) in his monograph of the family Ophryoscolecidae described *E. caudatum* Stein with a contractile vacuole situated near the dorsal side close to the anterior end of the macronucleus. He believed this species to be conspecific with *E. caudatum* Stein sensu Schuberg, and surmised that the last author had erroneously depicted the vacuole as being situated near the ventral body side. In 1930 Kofoid and MacLennan (18) described two new species of *Entodinium*—*E. laterale* and *E. rectangulatum*—both with the caudal spination similar to that of *E. caudatum* Stein but with the contractile vacuole situated "near the middle of the left body-side" on the level of the oesophagus. Thus, at present at least three species of *Entodinum* with the "caudatum" type of the caudal spination are known.

The taxonomic value of the caudal spination became still more limited when Poljansky and Strelkow (20, 21) showed that in *E. caudatum* this spination is environmentally plastic, the range of food-induced variability embracing forms of this spination, previously believed to be characteristic of different species of this genus. In clones, started with one individual of *E. caudatum* with a spination typical of this species, individuals soon appeared with a caudal spination typical of *E. loboso-spinosum*—with a dorsal spine and but one ventral lobe—as well as tailless individuals morphologically indistinguishable from "*Entodinium simplex* Dogiel". When host animals were fed herbs and hay, forms with reduced caudal spination predominated in the population; when food was rich in starch, forms with well developed caudal spination typical of *E. caudatum* became predominant.

In the material collected in Pakistan, three different forms of *Entodinium* with the "caudatum" type of caudal spination were present. They were accompanied by three corresponding forms with spination of the "loboso-spinosum" type; the latter were connected by intermediary forms to variants without caudal spination. The present paper is an attempt to describe these forms and to discuss their relationships.

Materials and Methods

The material (rumen contents of 68 Jamnapari goats and of 59 sheep) was collected in the abattoir in Rawalpindi Cantonment, Western Pakistan, in the period between April 1950 and June 1954. The collection had to be done at night, as the usual time of butchering in Pakistan is about midnight in summer and between 8 and 10 p.m. in winter. The technique of collection was as follows: the rumen contents were thoroughly mixed inside the intact rumen of the freshly slaughtered animal and a cut 3–4 in. long was made in its wall.

A wide-meshed tea strainer was pressed against the cut and the fluid, penetrating into the sieve, collected into a wide-mouthed pipette. The gastric contents were mixed with an equal volume of 20% formalin, the resulting mixture thus containing about 3.8–4% formaldehyde.

The material for temporary mounts was stained with Weigert's iodine. In preparing permanent slides Westphal's (30) method, modified by the author, was used; a few drops of previously well-shaken material were introduced into a centrifuge tube filled with distilled water and the tube centrifuged for 3 minutes at about 600 revolutions per minute. The supernatant fluid was discarded and the sediment washed again in a fresh portion of distilled water. After it was centrifuged for the second time the sediment was mixed with an equal volume of human serum. A drop of the suspension was placed on a slide and spread to the size of a circle about 18–20 mm. in diameter. The slides were placed in a horizontal position in a glass jar containing cotton soaked in undiluted formalin. In about 18–20 hours the films had coagulated but even a stay of from 3 to 4 days in formalin vapors did not damage the material. The coagulated films were hardened for 10 minutes in 70% alcohol, rinsed in water, and stained with Heidenhain's iron haematoxylin or Ehrlich's haematoxylin, Mayer's acid haemalum in Hoare's (17) modification, or with Gower's (14) carmine. A few films were counterstained with orange G. The best results were obtained with Mayer's haemalum and Gower's carmine. Drawings were made with the aid of a camera lucida or a drawing prism.

Terminology

The morphological terminology used in the present paper is identical with that of Dogiel, of Kofoid and MacLennan, and of other recent authors. It has to be emphasized, however, that earlier authors, particularly Stein (25) and Schuberg (24), used a different terminology; Schuberg, for instance, designated the nuclear body side of *Entodinium* as left side whereas today it is designated as dorsal side. It is obvious that formally identical statements, for example, "The caudal spine of *E. caudatum* is bent to the left", have different meanings when used by Schuberg on the one hand and by a contemporary author on the other.

Today, the term "body length" designates the distance from the anterior pole of the body to the anal opening, whereas Schuberg (24), in 1888, meant the distance from the anterior pole to the end of the longest caudal spine. Thus, the length to width relation, deduced from measurements given by Schuberg, cannot be compared directly (as Dogiel did in his monograph, p. 62) with the length to width relation based on the modern method of measuring. It is important to keep these differences in mind in studying the older literature on the Ophryoscolecidae.

In the present paper the terms "caudatum," "loboso-spinosum," and "dubardi" types will be used to designate forms of *Entodinium* produced by the intraspecific variability within different species of this genus. The term "caudatum" type will be used to designate variants with one dorsal spine and

two ventral lobes similar in the development of the caudal spination to *E. caudatum* as described by Stein (25), Schuberg (24), and Dogiel (11). By the term "loboso-spinosum" type will be designated variants with one dorsal spine and only one—the left—ventral lobe, thus similar in the type of their caudal spination to *E. loboso-spinosum* Dogiel. Tailless variants, similar to *Entodinium dubardi* Buisson will be designated as the "dubardi" type.

E. dubardi is characterized by the length to depth relation not exceeding 1.55, as are the tailless variants described in the present paper. We have not found tailless individuals with length to depth relation of over 1.7 (which, according to Dogiel, is characteristic of *E. simplex*) and consider, therefore, the term "simplex" type to be inappropriate for their designation.

Species and forms of *Entodinium* described in this paper are obviously closely related to both *E. caudatum* and species of the "laterale" group of this genus in the sense of Kofoid and MacLennan. All these forms, including *E. caudatum*, will be referred to in this paper as forms of the "caudatum–laterale" group of *Entodinium*.

Entodinium rectangulatum and Allied Forms

E. rectangulatum Kofoid and MacLennan, 1930 forma ***caudatum*** f. nova (Figs. 1–2)

Diagnosis

Body nearly rectangular in side view 35(23–47) μ long and 29(23–39) μ deep. Contractile vacuole on mid-line of left side of body, on the level of the micronucleus ventrally to the broad left lateral groove. Two ventral lobes and one dorsal spine. Dorsal spine approximately as long as the dorsoventral diameter, with laterally compressed basis and long, narrow distal part. Left ventral lobe fleshy, triangular, with anal opening at base of mediodorsal surface. Right ventral lobe a thin plate with a nearly rectangular apex.

Type host: Domestic sheep.

Type locality: Rawalpindi, Western Pakistan. Collected in June 1951.

Measurements shown in Table I are based on 100 specimens from the rumen of sheep No. 48. The ciliates measured were stained with Mayer's acid haemalum and mounted in Canada balsam.

TABLE I

DIMENSIONS IN μ OF *E. rectangulatum* F. *caudatum* FROM THE RUMEN OF SHEEP 48, BASED ON MEASUREMENTS OF 100 SPECIMENS MOUNTED IN CANADA BALSAM

	$M \pm m$	σ	V (%)	Observed limits of variation
Length	34.62±0.44	±4.42	12.77	23.0–47.0
Dorsoventral diameter	29.23±0.31	±3.06	10.46	23.0–39.0
Relative length	1.16±0.01	±0.12	10.04	0.9– 1.5
Length of macronucleus	21.32±0.38	±3.80	17.87	11.0–31.0
Diameter of oral disk	16.74±0.26	±2.62	15.78	10.0–24.0
Length of dorsal spine	35.09±0.39	±3.85	10.99	26.0–44.0
Length of left ventral lobe	11.24±0.19	±1.85	16.44	7.0–17.0
Length of right ventral lobe	8.69±0.15	±1.48	17.03	4.0–14.0

Description

Body nearly rectangular in side view, 1.16(0.9–1.5) dorsoventral diameters in length (Figs. 1–2). Oral area set nearly at right angles to the main body axis. Ventral surface nearly flat, dorsal slightly convex, more so in its posterior part. Posterior end truncated at right angles. Base of dorsal spine set in sagittal plane, laterally compressed, and plough-shaped; distal part produced in long, narrow, flexible thorn. Length of dorsal spine equal to or longer than the dorsoventral diameter (depth) and usually equal to body length. Left ventral lobe fleshy, triangular, with acute apex. Anal opening situated at base of its mediodorsal surface and continuing in caudal direction as a shallow furrow running along mediodorsal surface of the lobe and nearly reaching its apex. Right ventral lobe a thin plate with a nearly rectangular tip.

Left lateral groove triangular with its apex situated between the micronucleus and the contractile vacuole just posterior to base of the outer adoral lip. Ventral boundary of left lateral groove formed by dorsal edge of left ventral lobe and a fold on the left lateral surface of the body which is the anteriad extension of this edge. Dorsal boundary produced by a cuticular fold running close to left ventral side of nuclear apparatus, from base of outer adoral lip caudad and reaching the dorsal side of the base of the dorsal spine on the level of the anus. Base of triangular left lateral groove, which is produced by a line connecting the anus with the dorsal edge of the dorsal fold on the level of the anus, usually equals half the dorsoventral diameter.

Mouth an ellipsoidal opening with its long axis set transversally. Oesophagus situated to the right from the anterior pole of the endoplasmic sac; bent dorsad, terminating in vicinity of the micronucleus; boundary layer distinct. Rectum begins at left side of posteroventral surface of endoplasmic sac; terminates in anal opening at base of left ventral lobe.

Macronucleus club-shaped with a broad anterior part; dorsal surface closely applied to body wall; its anterior end nearly reaches the bottom of outer adoral furrow. Length of macronucleus variable, but usually does not exceed the dorsoventral diameter. Ellipsoidal micronucleus situated near boundary between the middle and anterior third of macronucleus to left of sagittal plane.

Contractile vacuole situated near mid-line of left lateral surface ventrally to anterior end of left lateral groove. Center of vacuole in side view always projected ventrally to anterior part of oesophagus. Food consists of chloroplasts, starch granules, plant debris, yeasts, and bacteria.

Table I summarizes the results of measurements of 100 specimens of *E. rectangulatum* forma *caudatum*, taken at random from the faunule of the rumen of sheep No. 48. The specimens measured were stained with Mayer's acid haemalum and mounted in Canada balsam.

Relationship

E. rectangulatum f. *caudatum* is similar in the development of its caudal spination to *E. caudatum* Stein, 1859, *E. acutonucleatum* Kofoid and

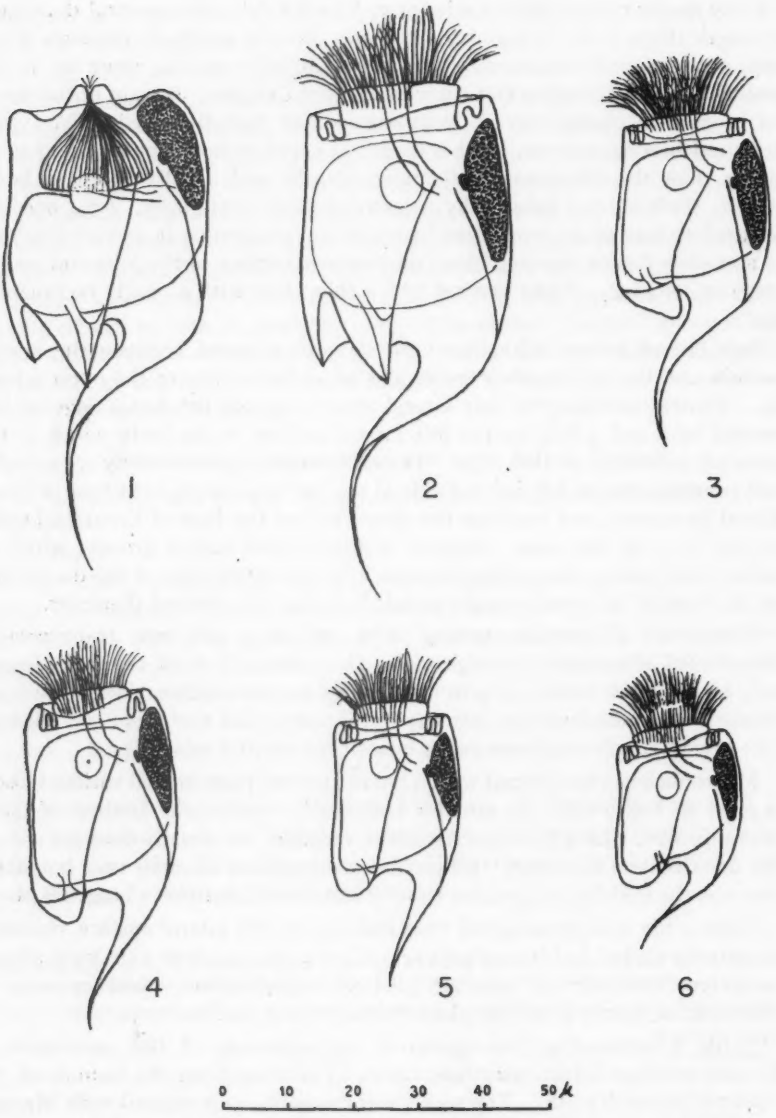


FIG. 1. *E. rectangulatum* forma *caudatum* forma nova (contracted specimen).
FIG. 2. *E. rectangulatum* forma *caudatum* forma nova (expanded specimen).
FIGS. 3–6. *E.* cf. *loboso-spinosum* Dogiel, 1927.
All specimens from the faunule of sheep No. 48.

MacLennan, 1930, and *E. ovoidonucleatum* Das Gupta, 1935 (7). (*E. ovoidonucleatum* is probably an exconjugant of *E. caudatum*.) *E. rectangulatum* f. *caudatum* differs from all these species in the position of its contractile vacuole. The lateral position of this vacuole in the new form of *E. rectangulatum* is its constant character. We have seen no individuals of this form with the vacuole situated near the nucleus. Such a position is improbable because it would involve the contractile vacuole being situated at the bottom of the left lateral groove. Thus the new form resembles both hitherto known species of the "laterale" group of *Entodinium*—*E. laterale* and *E. rectangulatum*—in the position of its contractile vacuole. The dimensions of *E. rectangulatum* f. *caudatum* nearly coincide with those of the typical form, except that the dorsal spine is much longer in the new form: 35(26–44) μ as compared to 6(4–8) μ in the typical form (see Table II). The slight difference in the body length may be correlated with the development of the caudal spination. It is well known that within a given species the grade of development of this spination is negatively correlated with the body length. It is remarkable that in our material we have been unable to find the typical short-spined form of *E. rectangulatum*. The length of the dorsal spine of the new form is extreme—it averages 101.2% of the body length (see Table I), the corresponding figures for the races "*A*" and "*B*" of *E. caudatum* described by Poljansky and Strelkow (21) being 83.5 and 53.5% of the body length, respectively.

The morphological similarity between *E. rectangulatum* and *E. caudatum* leads one to expect that the variability of these two species will also be similar. We were prepared to find in *E. rectangulatum* a polymorphism, expressed mainly in the high continuous variability of development of the caudal spination. And indeed, in the faunule of sheep No. 48 we found numerous forms of *Entodinium* of the "loboso-spinosum" type with the lateral position of the contractile vacuole similar to that of *E. rectangulatum*. But there were no transitional forms between the *Entodinum* of the "loboso-spinosum" type and the *E. rectangulatum* f. *caudatum*, the variation was not continuous. Moreover, the specimens of the "loboso-spinosum" type were numerous, while in the populations of *E. caudatum* the "loboso-spinosum" forms are usually scarce. The *Entodinium* of the "loboso-spinosum" type, found in the faunule of sheep

TABLE II

COMPARISON OF DIMENSIONS IN μ OF *E. rectangulatum* KOFOID AND MACLENNAN, 1930 AND *E. rectangulatum* F. *caudatum* F. NOVA

	E. rectangulatum after Kofoid and MacLennan	*E. rectangulatum* f. *caudatum*
Body length	35 (29–45)	35 (23–47)
Relative length	1.26 (1.1–1.5)	1.16 (0.9–1.5)
Dorsoventral diameter	28 (23–35)	29 (23–39)
Length of macronucleus	22 (14–30)	21 (11–31)
Diameter of oral disk	15 (12–19)	17 (10–24)
Length of dorsal spine	6 (4–8)	35 (26–44)

No. 48, as well as in other sheep, was very similar to *E. loboso-spinosum* Dogiel. As in Dogiel's description of this species (9, 11), the position of the contractile vacuole was not mentioned, it is impossible to decide whether the *Entodinium* of the "loboso-spinosum" type found by us is identical with, or different from Dogiel's species. We shall, therefore, discuss our form under the name *E.* cf. *loboso-spinosum* Dogiel, 1927.

E. cf. *loboso-spinosum* Dogiel, 1927* (Figs. 3–6)

Diagnosis

Body rectangular in side view, 26(18–33) μ long and 20(13–25) μ deep. Contractile vacuole on mid-line of left side of body, on level of the micronucleus ventrally to the broad left lateral groove. One ventral lobe and one dorsal spine. Dorsal spine long with laterally flattened basis and narrow, long, distal part. Left ventral lobe fleshy and rounded; in big specimens roughly triangular in side view, covering anus from the left side, but in small specimens ventral lobe small, rounded, not covering the anus. Right ventral lobe absent. The measurements given in Table III are based on 100 specimens from the faunule of sheep No. 48. The ciliates were stained with Mayer's acid haemalum and mounted in Canada balsam.

Description

Body nearly rectangular in side view, 1.3(1.1–1.7) dorsoventral diameters in length. Oral area set nearly at right angles to the body axis. Ventral surface nearly flat, dorsal slightly convex, more so in its posterior part. Posterior end truncated nearly at right angles.

Base of dorsal spine set in sagittal plane, laterally compressed and plough-shaped; distal part produced in a long, narrow, flexible spine. Dorsal spine usually shorter than the dorsoventral diameter. Left ventral lobe fleshy with a rounded apex; size in small specimens relatively shorter than in large. Right ventral lobe absent.

Left lateral groove triangular with apex situated between micronucleus and contractile vacuole near base of outer adoral lip; its ventral and dorsal boundaries less prominent than in *E. rectangulatum* f. *caudatum*. Base of triangular left lateral groove, that is, the line connecting its ventral and dorsal edge on the level of the anus, about half the length of the dorsoventral diameter.

Mouth, endoplasmic sac, and rectum similar to those of *E. rectangulatum* f. *caudatum*.

Macronucleus club-shaped, with a broad anterior part, nearly triangular in side view; closely applied to dorsal body wall and nearly reaching outer adoral furrow with its anterior end; length usually does not exceed dorsoventral diameter. Ellipsoidal micronucleus situated near boundary between the anterior and middle third of macronucleus to left of sagittal plane.

**E. loboso-spinosum* was described by Dogiel (9) in 1925 as a form of *E. dubardi* under the name *E. dubardi* forma *loboso-spinosum* and promoted by him to the rank of species in 1927 (11).

Contractile vacuole situated near middle of left body surface ventrally to left lateral groove on level of micronucleus; although its position varies slightly it never infringes on the ventral edge of the left lateral groove.

Food consists of bacteria and yeasts. The measurements of 100 specimens of *E.* cf. *loboso-spinosum* from the faunule of the rumen of sheep No. 48 are summarized in Table III. The stained specimens measured were mounted in Canada balsam. Their dimensions closely resemble those of *E. laterale* Kofoid and MacLennan, 1930 as shown in Table IV.

Relationship

Entodinium cf. *loboso-spinosum* closely resembles *E. loboso-spinosum* Dogiel, but differs slightly from it in its size and proportions (see Table V).

The outline of *E.* cf. *loboso-spinosum* is similar to that of *E. loboso-spinosum* depicted by Dogiel (11, Fig. 27*a*, p. 60).

TABLE III

DIMENSIONS IN μ OF *E.* CF. *loboso-spinosum*, FROM THE RUMEN OF SHEEP 48, BASED ON MEASUREMENTS OF 100 SPECIMENS MOUNTED IN CANADA BALSAM

	$M \pm m$	σ	V (%)	Observed limits of variation
Body length	26.40±0.34	±3.39	12.84	18–33
Dorsoventral diameter	19.68±0.22	±2.24	11.32	13–25
Relative length	1.34±0.01	±0.11	8.21	1.1–1.7
Length of macronucleus	13.32±0.25	±2.53	19.00	6–20
Diameter of oral disk	10.93±0.16	±1.62	14.86	6–15
Length of dorsal spine	16.39±0.20	±2.05	12.40	12–22
Length of ventral lobe	3.53±0.12	±1.18	33.14	0.7–7.0

TABLE IV

COMPARISON OF DIMENSIONS IN μ OF *E.* CF. *loboso-spinosum* DOGIEL, 1927 AND *E. laterale* KOFOID AND MACLENNAN, 1930

	E. laterale	*E.* cf. *loboso-spinosum*
Body length	25 (19–28)	26 (18–33)
Relative length	1.28 (1.5–1.55)	1.34 (1.1–1.7)
Dorsoventral diameter	19 (18–21)	20 (13–25)
Length of macronucleus	13 (8–18)	13 (6–20)
Diameter of oral disk	11 (9–14)	11 (6–15)

TABLE V

COMPARISON OF DIMENSIONS OF *E.* CF. *loboso-spinosum* AND *E. loboso-spinosum* DOGIEL, 1927

Species	Length	Relative length
Entodinium cf. *loboso-spinosum* Dogiel, 1925	26 (18–33)	1.34 (1.1–1.7)
E. loboso-spinosum Dogiel, 1925 from *Bubalus cokei* (after Dogiel)	33 (30–35)	1.57
E. loboso-spinosum Dogiel, 1925 from cattle (after Dogiel)	39 (30–46)	1.50

Dogiel states that both ventral and dorsal surfaces of *E. loboso-spinosum* are convex, but his figure (11, Fig. 27*a*) shows an *Entodinium* with a nearly flat ventral surface closely resembling in its outline *E.* cf. *loboso-spinosum*. The differences in dimensions and proportions of *E.* cf. *loboso-spinosum* and *E. loboso-spinosum* Dogiel probably lie within the limits of individual variation. The position of the contractile vacuole in "*E. dubardi* f. *loboso-spinosum*," described by Dogiel in 1925, is not mentioned in this description, nor is it in his monograph of 1927, in which this "forma" has received the rank of a species. It is, therefore, impossible either to differentiate or to identify *E.* cf. *loboso-spinosum* with Dogiel's *E. loboso-spinosum* without a re-examination of the type specimens of the latter species.

Occurrence

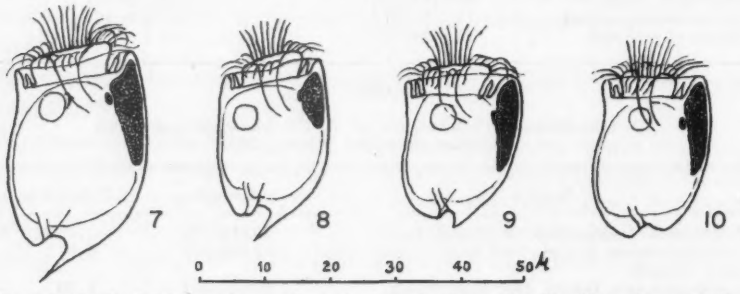
E. cf. *loboso-spinosum* was present in 49 out of 68 goats and 38 out of 59 sheep examined.

The "dubardi" Forms of E. cf. loboso-spinosum

Tailless forms of *E.* cf. *loboso-spinosum* were not found in the faunule of sheep No. 48 but were present in faunules of other sheep and goats. Of these, the faunule of Jamnapari goat No. G59 was of special interest. In it, *E.* cf. *loboso-spinosum* was the sole species of *Entodinium* with the lateral position of the contractile vacuole. The range of variability of its caudal spination embraced short-tailed typical forms on the one hand and tailless forms of the "dubardi" type on the other, with a continuous series of intermediary variants between them (Figs. 7–10).

The position of the contractile vacuole in these forms varies slightly; in some specimens it is situated near the ventral margin of the oesophagus (Figs. 9–10), in others it is shifted further ventrad, being situated in some individuals halfway between the level of the oesophagus and the ventral side of the body (Figs. 7–8). In these last forms the position of the contractile vacuole is similar to that in *E. caudatum* depicted by Schuberg (24).

The left lateral groove is present in the spinose forms but becomes progressively shallower as the length of the caudal spination decreases, and is not discernible in the tailless forms of the "dubardi" type.



FIGS. 7–10. *E.* cf. *loboso-spinosum* from the faunule of goat No. 59. Note the variability of the caudal spination and the position of the contractile vacuole ventrally to the level of the oesophagus.

Entodinium simulans and Its Forms

E. ***simulans*** sp. n., forma ***caudatum*** f. nova (Figs. 11, 12, 17)

Diagnosis

Body ovoidal in side view, 35(27–44) μ long and 28(21–34) μ deep. Contractile vacuole on mid-line of left side of body on level of micronucleus ventrally to left lateral groove. Left lateral groove narrow, long, slit-shaped in its anterior half. Its anterior end extends to distal margin of outer adoral lip. Oral disk slanted to left. Outer adoral lip very low in its left ventral part. Two ventral lobes and one dorsal spine. Dorsal spine much shorter than dorsoventral diameter. Left ventral lobe fleshy, triangular, with anal opening at its base. Right ventral lobe rounded with acute apex. The description and measurements compiled in Table VI are based on 100 stained and mounted specimens from the rumen of sheep No. 54.

Type host: Domestic sheep.

Type locality: Rawalpindi, Western Pakistan. Collected in June 1951.

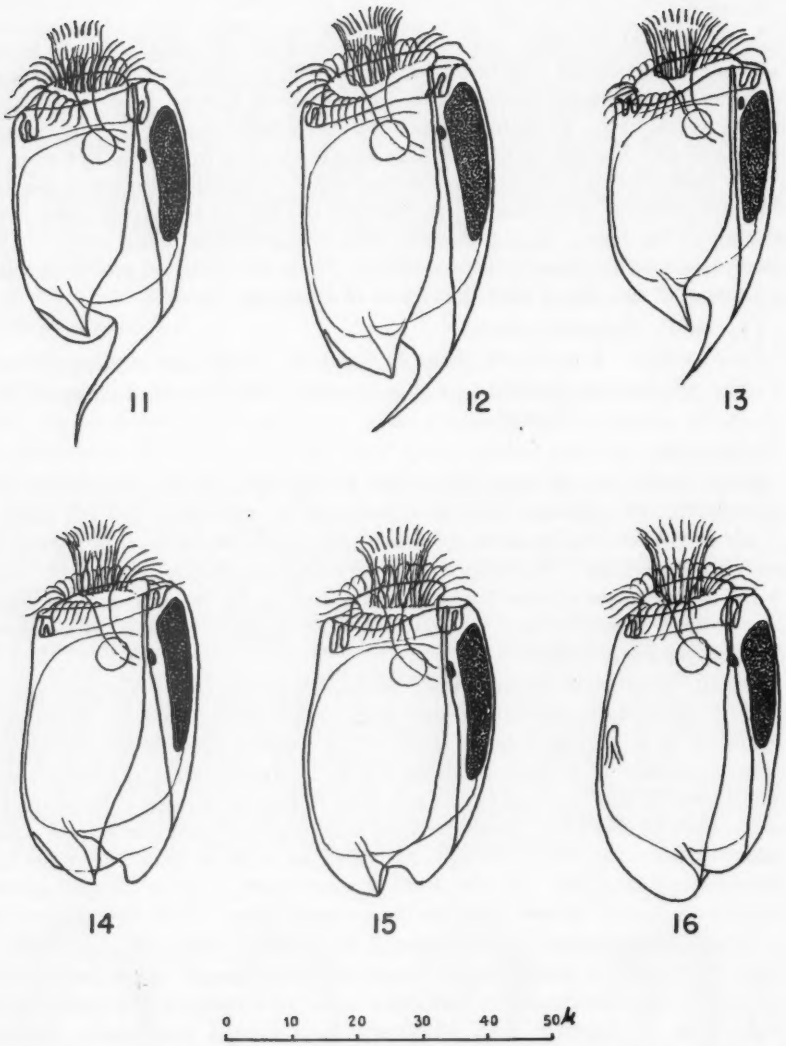
Type specimens: Institute of Parasitology, Macdonald College, Que., Canada.

Description

Body ovoidal in side view, 1.28(1.0–1.5) dorsoventral diameters in length. Oral disk in side view set nearly at right angles to main body axis but slanted to left so that the free base of the other adoral cilia lies on level of bottom of outer adoral furrow. Outer adoral lip very low in its left anterior part; apex of left lateral groove extending as narrow furrow to anterior edge of outer adoral lip. Posterior end of body slightly tapered, ventral lobes being directed posterodorsally.

Dorsal spine much shorter than dorsoventral diameter; its base set in sagittal plane, laterally compressed and plough-shaped and its distal part produced in a narrow flexible spine. Left ventral lobe fleshy, triangular, slanted dorsally at an angle of about 30°–40°. Anal opening situated at base of left ventral lobe and continuing as a shallow furrow running in the direction of the apex of this lobe. Right ventral lobe rounded, with acute apex. Left lateral groove narrow and long; its width at level of anus less than half dorsoventral diameter. At the level of micronucleus the left lateral groove tapers to a narrow furrow, reaching the anterior margin of the outer adoral lip.

Mouth an ellipsoidal opening slanted to the left. Oesophagus situated to right from anterior pole of endoplasmic sac, bent dorsad and terminating in vicinity of micronucleus; its boundary layer very distinct, the dark-stained endoplasm contrasting with the very light-stained ectoplasm. Rectum begins from left side of posteroventral surface of endoplasmic sac and terminates in the anal opening at base of left ventral lobe. Macronucleus club-shaped with a broad anterior end, nearly triangular in side view, closely applied to dorsal body wall and reaches the outer adoral furrow. Ellipsoidal micronucleus situated near boundary between anterior and middle thirds of the macronucleus.



FIGS. 11–16. *E. simulans* sp. n. from the faunule of sheep No. 54. FIGS. 11–12—the "caudatum" type; FIG. 13—the "loboso-spinosum" type; FIGS. 14–16—spineless forms with varying degrees of development of the caudal lobes.

TABLE VI

DIMENSIONS IN μ OF *E. simulans* SP. N., F. *caudatum* F. NOVA FROM THE RUMEN OF SHEEP 54, STAINED AND MOUNTED IN CANADA BALSAM

	$M \pm m$	σ	V (%)	Observed limits of variation
Body length	35.47±0.38	±3.82	11.07	27–44
Dorsoventral diameter	27.62±0.28	±2.80	10.14	21–34
Relative length	1.28±0.01	±0.10	7.81	1.0–1.5
Length of macronucleus	22.57±0.30	±3.00	13.29	16–29
Diameter of oral disk	14.85±0.15	±1.49	10.04	10–18
Length of dorsal spine	17.05±0.35	±3.52	20.64	10–27
Length of left ventral lobe	6.86±0.11	±1.12	16.33	2–10
Length of right ventral lobe	6.65±0.13	±1.29	19.40	3–10

Contractile vacuole situated at level of micronucleus ventrally to the left lateral groove; in left side view projected on the anterior part of oesophagus and anterior pole of endoplasmic sac.

Food consists of bacteria and yeasts. Spores of fungi and fragments of mycelium as well as starch granules have occasionally been seen in the endoplasmic sac.

Measurements, summarized in Table VI, were taken from 100 stained and mounted specimens of *E. simulans* from the rumen of sheep No. 54.

Occurrence

E. simulans was found in the faunules of 30 out of 59 sheep and 38 out of 68 Jamnapari goats examined.

Relationship

In its caudal spination and the position of the contractile vacuole *E. simulans* is similar to *E. laterale* and *E. rectangulatum* described by Kofoid and MacLennan in 1930 (18). It differs from both in having a very long and narrow, slit-shaped left lateral groove. The extension of this groove on the outer adoral lip is a peculiarity not described in any other species of *Entodinium*.

It has to be noted that *E. simulans* in the faunule of sheep No. 54, as well as in other faunules, exhibited a continuous variation in the development of the caudal spination similar to that of *E. caudatum*. Two forms, connected by transitional variants, could be distinguished—*E. simulans* f. *loboso-spinosum* and *E. simulans* f. *dubardi*; these forms are described below.

E. ***simulans*** sp. n., forma ***loboso-spinosum,*** forma n. (Fig. 13)

Diagnosis

Similar to *E. simulans* f. *caudatum* except in the development of the right ventral lobe, which is absent or reduced to a narrow cuticular fold without pointed end. The left ventral lobe may be pointed as in the "caudatum" form or rounded. This form was very rare in the faunule of sheep No. 54. The measurements recorded in Table VII are based on seven specimens from this faunule.

TABLE VII

DIMENSIONS IN μ OF SEVEN SPECIMENS OF *E. simulans* SP. N., F. *loboso-spinosum* F. NOVA FROM THE RUMEN OF SHEEP 54, STAINED AND MOUNTED IN CANADA BALSAM

	Observed mean and limits of variation
Length	38 (25–46)
Dorsoventral diameter	27 (22–34)
Relative length	1.36 (1.10–1.50)
Length of macronucleus	17 (12–23)
Diameter of oral disk	14 (11–16)
Length of dorsal spine	14 (10–17)
Length of ventral lobe	7 (6– 9)

E. ***simulans*** sp. n. forma ***dubardi*** forma n. (Figs. 16, 18)

Diagnosis

Similar to *E. simulans* f. *caudatum* and *E. simulans* f. *loboso-spinosum* except in the development of the caudal armature. Spines absent, right ventral lobe absent. Left ventral lobe and caudal lobe short, rounded, without pointed angles. Left lateral groove as in *E. simulans* f. *caudatum* but sometimes shallow. Contractile vacuole always near the middle line of the left body side at a considerable distance from the nuclear apparatus. Measurements, summarized in Table VIII, are those of 100 stained specimens, mounted in Canada balsam, from the faunule of sheep No. 54.

E. simulans forma *dubardi* is a tailless form of *E. simulans* sp. n., analogous to forms of *E. caudatum* designated by Poljansky and Strelkow (20, 21) as "simplex". The grounds for designating these forms as "dubardi" forms

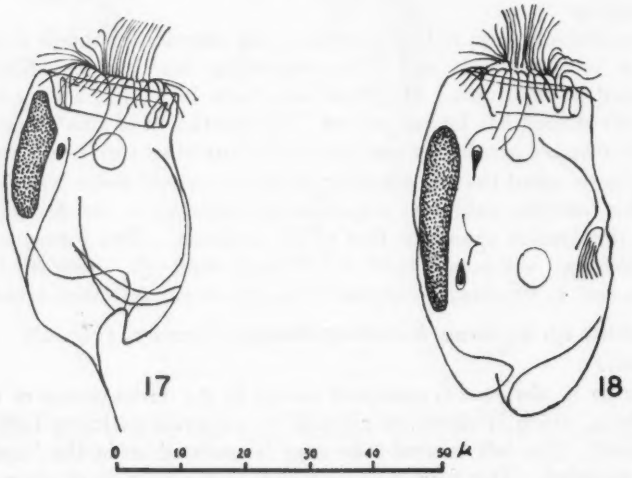


FIG. 17. *E. simulans* forma *caudatum* with a reduced right ventral lobe. Right side view.
FIG. 18. *E. simulans* forma *dubardi*. Right side view of a dividing specimen.

TABLE VIII

DIMENSIONS (IN μ) OF *E. simulans* F. *dubardi* FROM THE RUMEN OF SHEEP 54, BASED ON MEASUREMENTS OF 100 SPECIMENS, STAINED AND MOUNTED IN CANADA BALSAM

	$M \pm m$	σ	V (%)	Observed limits of variation
Length	31.92±0.57	±5.72	14.60	21–47
Dorsoventral diameter	23.04±0.35	±3.46	15.04	16–34
Relative length	1.37±0.01	±0.11	8.02	1.2–1.7
Length of macronucleus	17.87±0.36	±3.61	20.11	11–28
Diameter of oral disk	12.26±0.24	±2.42	19.66	8–20
Length of dorsal lobe	3.52±0.12	±1.21	34.38	1.3–8.6
Length of ventral lobe	6.05±0.12	±1.22	20.18	2.7–10.0

were mentioned in the section on materials and methods. Their further discussion necessitates an inquiry into the validity of Dogiel's species *E. simplex*.

In describing *E. simplex* Dogiel (10, 11) gave as the *sole* character distinguishing it from *E. dubardi* its relative length, namely 1.7–1.74 as compared with 1.55 in *E. dubardi*. On measuring Dogiel's (10, Fig. 2; 11, Figs. 3–4) figures, however, it is found that the length to width relation of the specimens depicted varies from 1.50 to 1.68 with an average of 1.62. The average is very close to that of 1.55 characteristic, according to Dogiel, of *E. dubardi* Buisson, 1923. In view of this *E. simplex* must be regarded as a synonym of *E. dubardi*.

A few years after the publication of Dogiel's monograph on the Ophryoscolecidae his associates, Poljansky and Strelkow (21), described a strain of what they call "*E. simplex* Dogiel". The relative length of "*E. simplex*" of their clone as summarized in their table 36 (21) varies from that given by Dogiel (11). Our recalculations of this table have given the following results: on September 11, 1934, the average length-to-width relation of *E. simplex* was 1.25 with limits of variation of from 1.06 to 1.56; on October 23, 1934, the average was 1.47 with limits of variation of from 1.28 to 1.67, and on December 23, 1934, the corresponding figures were 1.35, limits 1.04 to 1.64. It is hard to conceive why a strain of *Entodinium* with such characteristics should be identified as *E. simplex*. Poljansky and Strelkow's illustrations are in full agreement with the figures given in their tables. On measuring 10 specimens of "*E. simplex*" depicted by these authors in their Fig. 24*A* (21) we found the length to "width" relation to be 1.45 with limits of variation of from 1.33 to 1.53, the corresponding figures for their Fig. 23*B* being 1.55 (1.39 to 1.70), which is very close to the figures given by Dogiel (11) as characteristic of *E. dubardi*.

Describing the "simplex" forms of *E. caudatum* Poljansky and Strelkow (21) state:

> "quand *Ent. caudatum* est à l'état de "simplex" son phénotype ne diffère en rien de celui du vrai *Ent.* simplex".

Their corresponding illustration (Fig. 25) represents 10 specimens of the "*Ent. caudatum* a l'état 'simplex' " and gives an average length to width

relation of 1.52 with variations of 1.43 to 1.60, again figures characteristic according to Dogiel (11) of *E. dubardi*. It follows, therefore, that the "vrai *Ent. simplex*" of Poljansky and Strelkow is identical with *E. dubardi* Buisson, 1923, and that the tailless forms of their class VI of *E. caudatum* are phenocopies of *E. dubardi*, not of "*E. simplex*".

In their paper of 1938, Poljansky and Strelkow (21) state that the variants of class VI are similar to both *E. simplex* and *E. dubardi*, but that:

> "*Ent. dubardi* forma *dubardi* trouvé dans *Capreolus capreolus*, *Cervus canadensis* et les Antilopes d'Afrique, vue la difference de l'hôte, convient moin pour la comparaison."

Why this argument—the difference of hosts—should not be applicable to *E. simplex*, described by Dogiel (10) from the reindeer, is hard to understand.

As *E. simplex* Dogiel, 1925 is a synonym of *E. dubardi* Buisson, 1923, we have designated the tailless forms of *E. simulans* as forms of the "dubardi" type. This designation is the more appropriate as the relative length of these forms is close to that of *E. dubardi*.

E. caudatum Stein, 1859

E. caudatum Stein, 1859 sensu Dogiel, 1927 is similar to the above described species of *Entodinium* but differs from them in its more rounded body, larger size, shorter left lateral groove and, particularly, in the position of its contractile vacuole, which in this species is adjacent to the macronucleus. We found this species only once in sheep No. 2 from Rawalpindi (Figs. 19–24).

The faunule of this sheep consisted of only four species of the Ophryoscolecidae and of but one of the Isotrichidae. The numerical interrelations between individuals belonging to these species were as follows:

E. caudatum	93.0%
Entodinium longinucleatum	2.5%
Entodinium ovinum	2.5%
Polyplastron multivesiculatum	+
Isotricha prostoma	2.0%

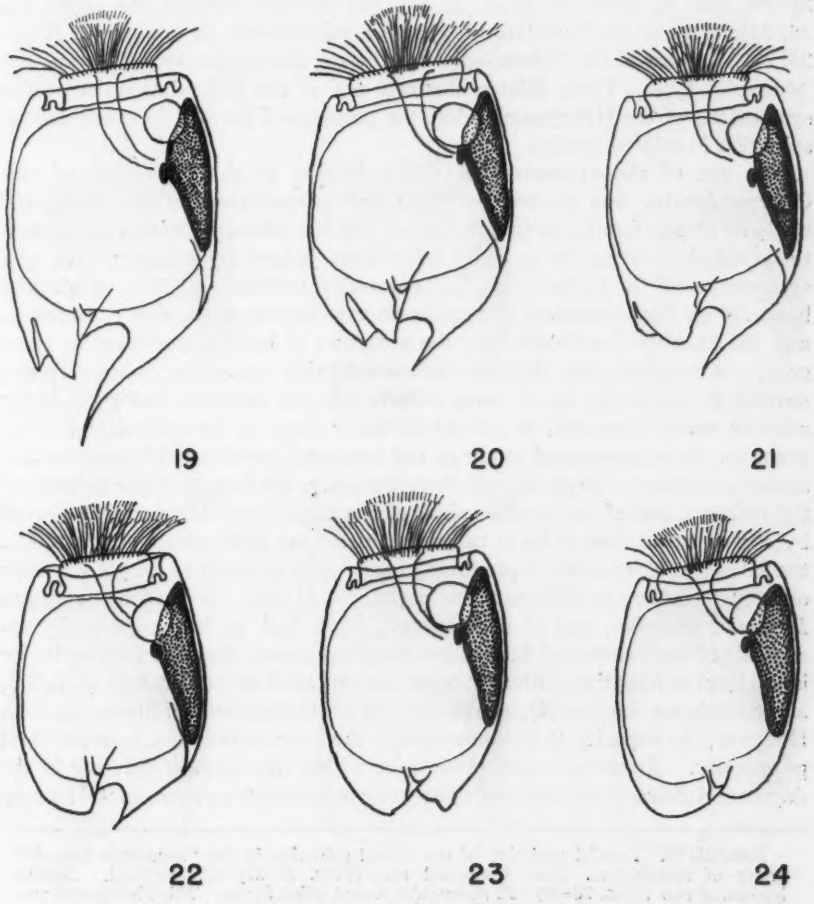
E. caudatum was represented by all six classes of development of the caudal spination, the continuous character of variation being immediately obvious. The distribution of individuals among the classes is shown in Table IX. The character of distribution was thus similar to that observed by Poljansky and Strelkow (21) in their clones of *E. caudatum*. The fourth class (that of individuals with caudal spination of the "loboso-spinosum" type) was

TABLE IX

DISTRIBUTION OF INDIVIDUALS OF *E. caudatum* AMONG THE CLASSES OF DEVELOPMENT OF THE CAUDAL SPINATION (MATERIAL FROM SHEEP 2)

Classes	I	II	III	IV	V	VI
Per cent individuals	9.0	4.5	1.5	0.5	4.0	80.5

represented by a few individuals only. In all classes the body length varied from 28 to 65 μ, but that of the tailed classes (I–V) only from 38 to 65 μ. The tailed individuals were thus, on an average, slightly larger than the tailless forms. The relation of length to depth varied from 1.1 to 1.7 with an average of from 1.37 ± 0.01. In individuals of the sixth (tailless) class this relation was 1.39 ± 0.01. The relation was thus closer to that of *E. dubardi* (1.55) than to that of Dogiel's "*E. simplex*" (1.70), as was the case in clones examined by Poljansky and Strelkow. The limit of variations of the relative length in the population of sheep No. 2 was also comparable to that observed in clones of *E. caudatum* studied by these authors.



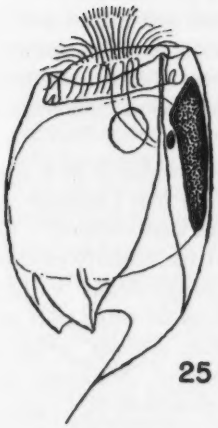
FIGS. 19–24. *E. caudatum* Stein, 1859 from the rumen of sheep No. 2. Specimens of the I–VI classes.

Discussion

Owing to the parallel variation of the caudal spination in *E. caudatum*, *E. rectangulatum*, and *E. simulans*, three different forms of the "caudatum" as well as of the "loboso-spinosum" and "dubardi" types were present in our material. These parallel forms, belonging to different species but possessing a common type of caudal spination, would have been identified on the basis of the characters of this spination as *E. caudatum*, *E. loboso-spinosum*, and *E. dubardi*, respectively. It is probable that, in the literature on the Ophryoscolecidae, parallel forms which belong to different species of *Entodinium* are concealed under these specific names. Figs. 25 to 33 represent such parallel forms of *E. simulans* (Figs. 25–27), *E. rectangulatum* (Figs. 28–30), and *E. caudatum* (Figs. 31–33). In the first vertical row (Figs. 25, 28, 31) forms of the "caudatum" type are represented, in the second (Figs. 26, 29, 32) those of the "loboso-spinosum" type, and in the third, forms of the "dubardi" type. These figures illustrate one of the main difficulties of the systematics of the Ophryoscolecidae, the presence of parallel forms of caudal spination in related species.

The use of the characters of this spination in the taxonomy of the Ophryscolecidae was started by Stein and perpetuated by all subsequent students of this family, including Kofoid and his school. This usage seemed to be simple and, as far as could have been judged by analogy with the systematics of the higher animals, reasonably reliable. Zoology originated basically on the foundation of studies on vertebrates and higher arthropods, and the practice has shown that the structure of body appendages in these groups is constant and, therefore, of considerable taxonomic value. It was natural to extend the use of these criteria into the realm of zoology of lower animals where, however, it proved in many cases to be misleading. For example, the experimental study of the temporal variation in Cladocera has shown that many morphological characters (e.g. the length of the helmet, of the rostrum, and of the caudal spine) of the members of this group, believed by early systematists to be of taxonomic value are environmentally variable, the intraspecific variability producing forms once believed to be good species or even to belong to different genera (28, 29, 31–33). Numerous species of *Daphnia*, *Bosmina*, and of other genera have had to be suppressed. An analysis of environmental factors involved has shown that the leading factor is the level of nutrition, although other factors, such as temperature (3, 5, 28), and turbulence of water (4), also participate in the causation of this variability. However, the capacity to cyclomorphosis, the norm of reaction, is determined genetically. Numerous races often exist within one species, differing in the degree and character of their morphogenetic responses to environmental factors.

FIGS. 25–33. Parallel variation of the caudal spination in the "caudatum–laterale" group of *Entodinium*. First horizontal row (FIGS. 25–27)—*E. simulans*. Second horizontal row (FIGS. 28–30)—*E. rectangulatum* and allied forms. Third horizontal row (FIGS. 31–33)—*E. caudatum*. Left vertical row (FIGS. 25, 28, 31)—forms of the "caudatum" type. Middle vertical row (FIGS. 26, 29, 32)—forms of the "loboso-spinosum" type. Right vertical row (FIGS. 27, 30, 33)—forms of the "dubardi" type.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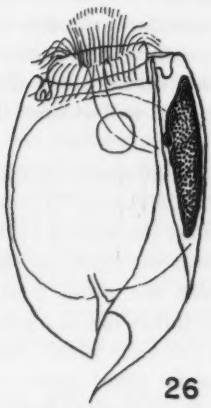
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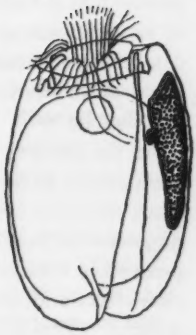
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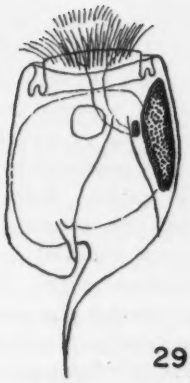
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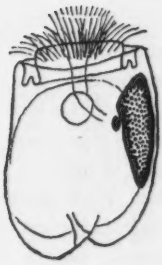
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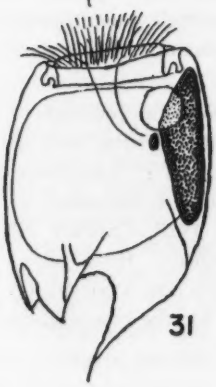
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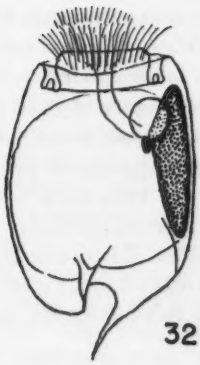
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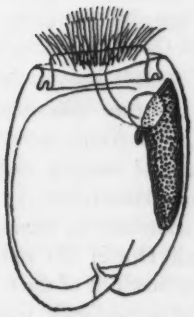
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Another example of confusion in systematics caused by pronounced environmental and genetical intraspecific variability is that of the well-known history of examination of the brine shrimp, *Artemia salina* (15). Similar examples can be drawn from the systematics of mollusks (e.g. *Ostrea*, *Mytilus*, *Vermetus*, *Anodonta*, and other genera), as well as from that of the annelids, rotifers, and dinoflagellates.

At the present time the systematics of the Ophryoscolecidae is in a state comparable to that of the Cladocera or Dinoflagellata over half a century ago when the basic concepts of the doctrine of cyclomorphosis were evolved. The diagnoses of many species of the Ophryoscolecidae and particularly of those possessing caudal armature are often based on intraspecifically variable characters and, as a result, forms, belonging to one species are often split into several. This is the case not only within the "caudatum–laterale" group of *Entodinium* but also in some other genera of this family. Poljansky and Strelkow (21) have shown that forms of *Eudiplodinium* described by Dogiel as *Eudiplodinium neglectum* forma *dilobum*, *Eudiplodinium neglectum* forma *monolobum*, and *Eudiplodinium neglectum* forma *bovis* and promoted to the rank of species by Kofoid and MacLennan (19) are environmentally induced modifications of one species and are not separate species, as these last authors presumed. On the other hand, it is almost certain that some other forms of *E. neglectum* described by Dogiel (e.g. *E. neglectum* forma *insigne*, *E. neglectum* forma *giganteum*), which also were promoted by Kofoid and MacLennan to the rank of species, really are valid species. Poljansky and Strelkow have shown that, while some species of the Ophryoscolecidae (e.g. *Entodinium caudatum*, *Eudiplodinium neglectum* forma *dilobum*) are extremely environmentally plastic, others (e.g. *Eudiplodinium maggii*, *Entodinium vorax**) are stable. Woltereck (33), who observed similar phenomena in Cladocera, has divided species which he examined into groups of stenotypic species with a low grade of intraspecific variability, and of schizotypic species with high intraspecific variability—terms which correspond roughtly to labile and stable species of Schmalhausen (23). In these terms the basic difficulty in the systematics of the Ophryoscolecidae can be defined as one of estimating the taxonomic values of morphological characters of labile species.

Another difficulty in the systematics of these ciliates lies in the presence of parallel forms in related species, forms which, when identified on the basis of parallel varying characters, are aggregated into one "species". Awerinzew and Mutafowa (1) as early as 1914 drew attention to the existence of parallelism of forms in the Ophryoscolecidae. Later, in 1923, Crawley (6) and Dogiel (8) published tables illustrating this parallelism. Both tables are now out of date. On the other hand, experimental data now available on the intraspecific variability in this family are inadequate for the compilation of an up-to-date table.

*These last two species have no caudal spination.

Parallelism of forms exists in other classes of Protozoa as well. Hoare (16), studying Eimeriidea from Uganda, has showed that:

> "When the genera comprising this suborder (with a few exceptions) are arranged in the ascending order of the number of structural elements (sporocysts and sporozoites) contained in their oöcysts, they form parallel series of groups in the horizontal and vertical directions, which consist of homologous members, as represented graphically. . . The oöcyst characters of each genus are determined by those of its neighbours in the horizontal and vertical groups."

In the graph shown by Hoare, vacant places were left for as yet undiscovered genera with predicted structures of oöcysts. At least three genera (Gouseff (13), Ray *et al.* (22), Vincent (27)) have since been described to fill the vacancies in Hoare's graph—a unique case of prediction of existence of new genera in zoological literature.

Parallelism of forms is a serious source of error in both zoological and botanical systematics and becomes of considerable importance in cases of discontinuous intraspecific variability. "Varietal alternative characters," states Vavilov (26), "were often mixed with those of radicals; particularly was this the case when descriptions of new genera and Linneons were made on single plants and samples collected in one district."

The systematics of the lower taxonomic units of the Ophryoscolecidae can be reconstructed only on the basis of experimental studies of the variability of their numerous species, and this may require decades of strenuous work. The lifetime efforts of many zoologists will probably be needed to conduct the systematics of these ciliates between the Scilla and Charybdis of environmental plasticity and of parallel variation to the safety of exact knowledge, based on experimental data.

A mitigation of the vices of present-day systematics in the case of the Ophryoscolecidae can be achieved, however, by a careful selection of characters used for the identification of their species. Strict distinction should be made whenever possible between intraspecifically variable characters ("Gestaltmerkmale" of Woltereck), and stable characters of taxonomic value (his "Taxonomische Merkmale").

The characters of the caudal spination in the "caudatum–laterale" group of *Entodinium* are beyond doubt "Gestaltmerkmale" in the sense of Woltereck. Stereotyped forms of the caudal spination—the "caudatum," "lobosospinosum," and the tailless "dubardi" types—are repeated in at least three species of this group, species differing from each other in such essential morphological characters as the development and shape of the left lateral groove and the position of the contractile vacuole. In *E. caudatum* the latter is situated near the anterior end of the macronucleus as it is in most species of this genus. The left lateral groove in *E. caudatum* is less developed than in *E. rectangulatum* and *E. simulans*. This compels us to regard *E. caudatum* as a primitive member of the "caudatum–laterale" group. In *E. rectangulatum* the contractile vacuole became displaced ventrad to the level of the oesophagus,

and the anterior end of the elongated left lateral groove has separated this vacuole from the nuclear apparatus. In *E. simulans* the elongation of the left lateral groove has resulted in its extension on the outer adoral lip. Simultaneously, the left lateral groove became transformed into a furrow. This compels us to regard *E. simulans* as an advanced member of the "caudatum–laterale" group.

Thus, within this group of *Entodinium* exists an evolutionary trend towards the ventrad displacement of the contractile vacuole and towards the transformation of the left lateral groove into a long narrow furrow extending to the adoral lips. The probable significance of these evolutionary trends in the macroevolution of the Ophryoscolecidae will be discussed in another paper.

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STUDIES ON THE EVOLUTION OF THE OPHRYOSCOLECIDAE (CILIATA: OLIGOTRICHA)

II. ON THE ORIGIN OF THE HIGHER OPHRYOSCOLECIDAE[1]

G. LUBINSKY

Abstract

A comparison of the structure of *Diplodinium* s. str. and *Entodinium* of the "caudatum–laterale" group leads to the conclusion that *Diplodinium* originated from *Entodinium* as the result of torsional displacement of the nuclear apparatus, the left lateral groove, and the contractile vacuole from the dorsal side to the ventral. The ventral side of *Entodinium* was thus transformed into the "dorsal" side of *Diplodinium* The probable influence of this new conception of the comparative morphology of the Ophryoscolecidae is discussed.

The family Ophryoscolecidae is divided into two groups: the lower Ophryoscolecidae, comprising one genus, *Entodinium*, and the higher Ophryoscolecidae, comprising all other genera of this family. *Entodinium* is characterized by the presence of only one membranelle zone (the adoral) of one contractile vacuole, and by the micronucleus situated ventrally to the macronucleus. The higher Ophryoscolecidae have two membranelle zones (the adoral and the dorsal) two or more contractile vacuoles, and the micronucleus situated dorsally to the macronucleus.

Without doubt the higher Ophryoscolecidae originated from ancestors similar to existing species of *Entodinium* (2, 3). According to Dogiel, the dorsal membranelle zone of *Diplodinium* s. str. (Dogiel's subgenus *Anoplodinium* of the genus *Diplodinium* s. lato) originated as the result of dorsad growth of the left outer end of the oral membranelle zone of *Entodinium*, which in the course of this growth became bent to the left and produced the semicircular adoral zone of *Diplodinium* s. str. Simultaneously, the contractile vacuole and the micronucleus were displaced dorsad and became situated dorsally to the macronucleus. Thus Dogiel takes it for granted that the dorsal side of the higher Ophryoscolecidae is homologous to the dorsal side of *Entodinium*. In 1946 (5)* and again in 1951 (7, p. 430) Dogiel depicted a hypothetical *Entodinium*, ancestral to the higher Ophryoscolecidae, with its micronucleus situated dorsally to the macronucleus—a character not observed in any existing species of this genus.

In 1923 Crawley (2) wrote:

> "It is impossible to avoid the conclusion that *Diplodinium* [s. lato: author] is derived from *Entodinium*. What apparently has happened is that the right and the left sides of the anterior surface of *Entodinium* have grown in, separating the originally large mouth into two

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*This picture is absent in the English version of Dogiel's paper (6).

> portions. Of these, the larger ventral portion has continued as a functional mouth, while the smaller dorsal portion has become reduced to a pit extending but a short distance into the interior. At the same time, the original single system of cirri has also separated into two, producing one crown, which encircles the mouth and another which encircles the pit . . . ".

Dogiel (4) correctly states that there is no pit† in the middle of the dorsal membranelle zone of *Diplodinium* and that this zone does not form a closed spiral. Thus the morphological characters required by the theory of Crawley do not exist in the genus *Diplodinium*.

A hypothetical reconstruction of the long series of ancestral forms connecting typical higher Ophryoscolecidae with typical primitive species of *Entodinium* is difficult. Morphological characters, which according to both Crawley and Dogiel should be present in ancestral forms of the higher Ophryoscolecidae (e.g. the dorsad growth of the oral membranelle zone and the displacement of the micronucleus to the dorsal side in *Entodinium*), are not found in existing species of this primitive genus. In the present paper an attempt is made to interpret the morphology of the primitive representatives of the higher Ophryoscolecidae on the basis of evolutionary trends observed in some higher representatives of *Entodinium*.

One of the most primitive genera of the higher Ophryoscolecidae is doubtless the genus *Diplodinium* s. str. in the sense of Kofoid and MacLennan (9). This genus lacks a skeleton, which is characteristic of all other genera of the higher Ophryoscolecidae except *Eodinium*. Another peculiarity of *Diplodinium* is the location of the nuclear apparatus not near the dorsal side, as is the case in all other genera of the Ophryoscolecidae, but near the middle of the right side of the body. A long narrow longitudinal furrow is situated between the nuclear apparatus and the contractile vacuoles of *Diplodinium* s. str. and *Eudiplodinium* sensu Kofoid and MacLennan. Any theory attempting to explain the origin of the higher Ophryoscolecidae must also explain the genesis of these peculiar traits of these two primitive genera of this group.

In studying the morphology of two species of *Entodinium* related to *E. caudatum* (that of *E. rectangulatum* Kofoid and MacLennan, 1930 and *E. simulans* Lubinsky, 1957 (10)) we have noted that in the last two species of *Entodinium*, there exists a trend to anteriad elongation of the left lateral groove, which in *E. simulans* becomes transformed into a long narrow furrow. The contractile vacuole in these species of *Entodinium* is shifted ventrad to the level of the mouth and becomes separated from the nuclear apparatus by the anterior portion of the elongated left lateral groove.

†(Crawley's opinion on the existence of such a pit was probably based on the incorrect drawings of Eberlein, who depicted such pits in *Eudiplodinium maggii* (= *D. maggii*) and *D. dentatum* (8, Plate 7, Figs. 8, 9, 17), and of Awerinzew and Mutafowa, who represented such pits in their figures of *Ostracodinium dentatum* (their "*D. dentatum*") and *Metadinium medium* (1, Plate 9, Figs. 1, 2, 7).

The comparison of interrelations of organelles on the *left* body side of *E. simulans* with those on the right body side of *Diplodinium*, for example, *D. dentatum* (Figs. 4, 6), reveals a striking similarity in their shape and sequence. In both cases they are (from left to right): contractile vacuole, longitudinal furrow, micronucleus, and macronucleus. Two alternative explanations can be given for this phenomenon:

1. As the longitudinal furrow of *Diplodinium* is situated on its *right* body side, whereas in *E. simulans* (and in other species of *Entodinium*) it is situated on the *left* body side, the furrow of *Diplodinium* is not homologous to the left lateral groove of *Entodinium*. The similarity in topographic interrelations of the nuclear apparatus, longitudinal folds, and contractile vacuoles in these two genera is a coincidence and of no significance.

2. The similarity of topographic relationships of the nuclear apparatus, longitudinal fold, and the contractile vacuoles in *Entodinium* of the "caudatum–laterale" group, on the one hand, and in *Diplodinium* s. str., on the other, is not accidental. The right lateral furrow of *Diplodinium* s. str. is homologous to the left lateral groove of *Entodinium*. The "right" body side of *Diplodinium* is homologous to the left body side of *Entodinium*.

The acceptance of the second hypothesis implies the following conception of the origin of the higher Ophryoscolecidae: Primitive representatives of the genus *Diplodinium* s. str. originated from ancestors similar to existing species of *Entodinium* of the "caudatum–laterale" group in such a way that the contractile vacuole, the furrow-shaped left lateral groove, and the nuclear apparatus were shifted ventrad across the left body side. The oesophagus followed the macronucleus. The apex of the left lateral furrow moved ventrad, separated the ventral membranelle row ("dorsal" zone) from the oral apparatus, and was transformed into the operculum. The ventral side of *Entodinium* became the "dorsal" side of *Diplodinium*.

Phylogenetic phenomena required by this conception are based on evolutionary trends observed in the "caudatum–laterale" group of *Entodinium*—trends towards the transformation of the left lateral groove into a narrow furrow and to the ventrad displacement of the contractile vacuole (10).

The following evolutionary steps can be traced from a study of existing species of the Ophryoscolecidae. Existing species similar to ancestral forms are noted after each step.

1. Primitive species of *Entodinium* with elongate body without caudal spination and lateral groove. *E. elongatum*, *E. nanellum* (Fig. 1), and others.

2. Origin of forms with caudal spination of the *E. caudatum* type possessing a wide and comparatively short left lateral groove. *E. caudatum* (Fig. 2).

3. Increase in length of the left lateral groove and ventrad shift of the contractile vacuole. *E. laterale* and *E. rectangulatum* (Fig. 3).

4. Left lateral groove becomes transformed into a narrow furrow, which extends to the anterior margin of the outer adoral lip and separates the contractile vacuole from the nuclear apparatus. *E. simulans* (Fig. 4).

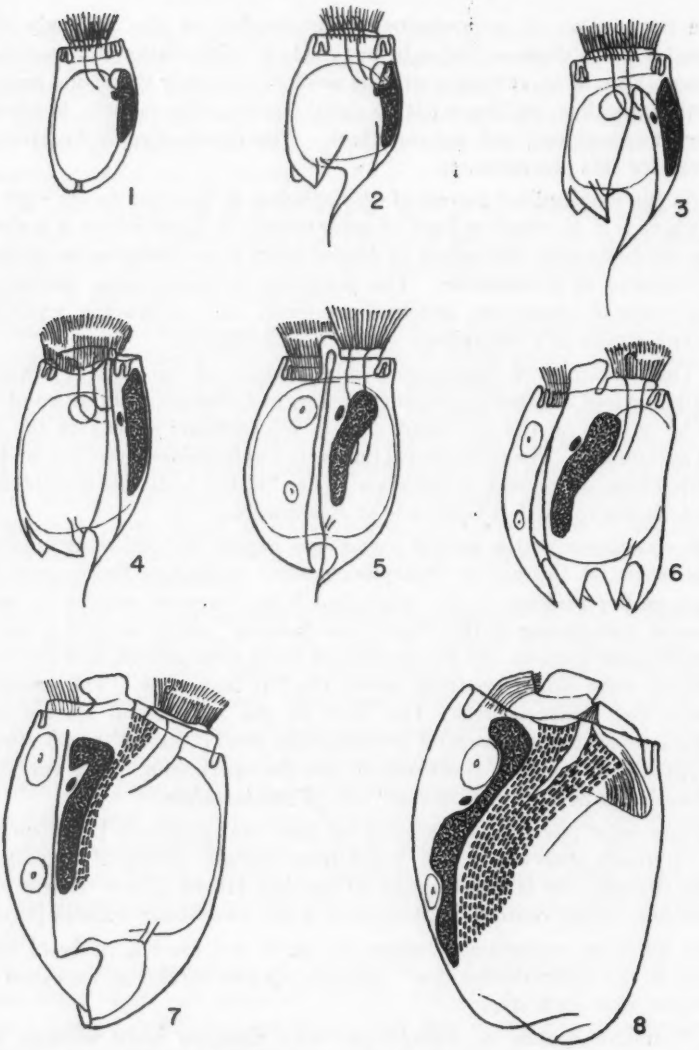

FIG. 1. *Entodinium nanellum* Dogiel, 1923 (× 800).
FIG. 2. *E. caudatum* Stein, 1859 (× 630).
FIG. 3. *E. rectangulatum* Kofoid and MacLennan, 1930 (× 630).
FIG. 4. *E. simulans* Lubinsky, 1957 (× 630).
FIG. 5. Hypothetical ancestor of the genus *Diplodinium*.
FIG. 6. *D. dentatum* (Stein, 1859) Schuberg, 1888 (× 450).
FIG. 7. *Eudiplodinium maggii* (Fiorentini, 1889) (× 330).
FIG. 8. *Metadinium tauricum* (Dogiel and Fedorowa, 1925) (× 275).

All figures except Fig. 5 are slightly schematized camera lucida drawings of fixed and mounted specimens of Ophryoscolecidae from sheep and goats from material collected by the author in Rawalpindi, Western Pakistan, in 1950–54.

Drawings are made at different magnifications in order to reduce the progressive increase in size from Fig. 1 to Fig. 8.

5. Further ventrad shift of the nuclear apparatus, left lateral furrow, and contractile vacuole. The fold, produced by the anterior end of the left lateral furrow on the outer adoral lip, moving ventrad, separates the ventral membranelle row ("dorsal zone") from the adoral membranelle zone and becomes the operculum. The oesophagus follows the macronucleus. Advent of second contractile vacuole. Missing link (Fig. 5).

6. Shifting further ventrad, the contractile vacuoles reach the ventral side of the body. As the angular velocity of rotation of the macronucleus is lower near the oral end, the macronucleus becomes flexed (boomerang-shaped). Left lateral furrow still persists. *Diplodinium* (Fig. 6).

7. Further ventrad shift of the nuclear apparatus. The "skeleton" appears on the track of the macronucleus. *Eudiplodinium* (Fig. 7) and *Eremoplastron.*

8. Disappearance of the left lateral furrow and pressing of the macronucleus between the contractile vacuoles. Further development of the skeleton. *Metadinium* (Fig. 8) and other genera of the higher Ophryoscolecidae.

The essential point of our conception of the origin of the higher Ophryoscolecidae is the transformation of the ventral side of *Entodinium* into the "dorsal" side of *Diplodinium* as a result of the ventrad shift of the vacuoles, left lateral groove, and the nuclear apparatus. These phenomena can be described as torsional displacement of these structures across the left side of the body. This torsion occurred early in the evolution of the Ophryoscolecidae and resulted in the advent of the higher genera of this family. The stages of this process are shown in Figs. 1 to 8 in which, however, all drawings, with the exception of Fig. 5, are camera lucida drawings of existing species of the Ophryoscolecidae. Fig. 5 represents a hypothetical ancestor of the higher Ophryoscolecidae. The comparison of the left side view of *E. simulans* with the "right" side view of *D. dentatum* (Figs. 4, 6) makes the gap between the genera *Entodinium* and *Diplodinium* appear much smaller than it is usually believed to be. These phylogenetic phenomena can be compared with the processes of torsion (with subsequent detorsion) frequently occurring in the life cycle of apostomatous ciliates. Indeed, the figures representing the formation of the hypertomite in *Phoretophrya nebaliae* (11) may serve as a good illustration of the origin of the genus *Diplodinium.*

In terms of the usual terminology the following table of homologies between *Entodinium* and *Diplodinium* can be compiled:

Entodinium	*Diplodinium*
Dorsal side	Ventral side
Left side	Right side
Ventral side	Dorsal side
Right side	Left side
Left lateral groove	Right cuticular furrow
Dorsal spine	Ventral spine

The last point seems to be of special interest in regard to some aspects of the problem of morphological parallelisms in the family Ophryoscolecidae. In discussing this problem Dogiel (4, p. 280) states that there is no parallelism in the structure of caudal spination in three-spined species of *Entodinium* on

the one hand and three-spined species of *Diplodinium*, *Epidinium*, and *Caloscolex*, on the other because in *Entodinium* the longest spine is always the dorsal whereas in *Diplodinium*, *Epidinium*, and *Caloscolex* it is the ventral. From our standpoint, however, the so-called ventral spine of the higher Ophryoscolecidae is a homologon of the dorsal spine of *Entodinium*. Accordingly, the facts which Dogiel used to disprove the presence of parallelism between *Entodinium* and the higher Ophryoscolecidae we use as a proof of its existence.

The new conception of the origin of the higher Ophryoscolecidae may provide a new approach to the evaluation of morphological parallelisms in this family and probably a new basis for the study of the comparative morphology of the group. An attempt to reconstruct the phylogeny of the family Ophryoscolecidae on the basis of the new conception will be made in a subsequent paper.

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STUDIES ON THE EVOLUTION OF THE OPHRYOSCOLECIDAE (CILIATA: OLIGOTRICHA)

III. PHYLOGENY OF THE OPHRYOSCOLECIDAE BASED ON THEIR COMPARATIVE MORPHOLOGY[1]

G. LUBINSKY

Abstract

The leading trend in the evolution of the family Ophryoscolecidae was the torsional displacement of the nuclear apparatus from the primary nuclear side ("dorsal" side of *Entodinium*) to the secondary nuclear side ("dorsal" side of the higher Ophryoscolecidae). This shift was accompanied by progressive development of the skeleton and by polymerization of organelles. Creation within the family Ophryoscolecidae of three new subfamilies, based on the grade of torsional displacement of structures and on some other characters, is proposed.

Introduction

In the previous paper of this series (21) we came to the conclusion that the higher Ophryoscolecidae originated from ancestors similar to *Entodinium simulans* as the result of torsional displacement of the nuclear apparatus, the slit-shaped left lateral groove, and the contractile vacuole from the dorsal side to the ventral. In the present paper an attempt is made to reconstruct the phylogeny of the Ophryoscolecidae on the basis of this conception.

The literature on the phylogeny of the Ophryoscolecidae consists of two papers, that of Crawley (2), published in 1923, and that of Dogiel, published in 1946 (7) in Russian and in 1947 (8) in English. Crawley summarizes his conception of the evolution of the Ophryoscolecidae as follows:

> "Generic distinctions are based on the form of the ciliary apparatus; specific—on the size and nature of the armature which is present on the posterior end of nearly all the species. These two sets of characters vary independently thus bringing about the unusual phenomenon of a change of genus without any change of species. This is shown with almost the precision of a mathematical demonstration for *Entodinium* and *Diplodinium*, and would probably also be shown in the same way for *Diplodinium* and *Metadinium* were our knowledge of this last genus as extensive as that of the other two."

A detailed criticism of Crawley's paper seems out of place here but it may be stated that he has confused intraspecifically variable characters—those of the caudal spination—with specific ones and on this misinterpretation based his opinion on the "change of genus without any change of species".

Dogiel's (7, 8) papers are concerned with the "Phylogeny of stomach-infusorians of ruminants in the light of paleontological and parasitological data." Analyzing the host-specificity of different genera and species of the

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Ophryoscolecidae, he comes to the conclusion that the genera *Entodinium* and *Anoplodinium* probably originated in the Eocene; *Epidinium* and *Eudiplodinium* in the Oligocene; *Ostracodinium* in the Miocene; *Caloscolex*, *Opisthotrichum*, *Ophryoscolex*, and *Polyplastron* in the Pliocene. He stated that:

> "different genera of Ophryoscolecidae may be very easily arranged in a gradually ascending line of progressive evolution, illustrated by several systems of organelles. In this way the ciliary apparatus, originally consisting of a simple oral zone, becomes completed by another, dorsal zone; the number of contractile vacuoles gradually increases; skeletal plates, primarily wanting, later appear and increase in number and size; the posterior end of the body gradually develops a more and more complex array of caudal spines."

Dogiel further distributes the genera of the Ophryoscolecidae among five "steps of the evolutionary ladder," but does not discuss the interrelations between genera. The aim of the present paper is to construct a dendrogram of the family Ophryoscolecidae on the basis of morphological data. This, however, necessitates a revision of some basic concepts of their morphology.

Displacement of Structures and the Morphological Terminology

In comparing genera and species of the Ophryoscolecidae, it is necessary to use terms designating their body sides and body surfaces. In 1895 Eberlein (10) proposed to designate as the "dorsal" side the nuclear side of the Ophryoscolecidae. Dogiel, as well as Kofoid and his school, accepted this definition without questioning the homology of the nuclear side of the higher Ophryoscolecidae to that of *Entodinium*. In 1925 Dogiel (4) wrote:

> "Je rapporte le côté à macronucleus de l'Entodinium au côté macronucleaire des Diplodinium aplatis, c'est-à-dire au côté dorsal."

Five years later Kofoid and MacLennan (16) stated that:

> "The side of the body (of *Entodinium*) marked by the macronucleus, is considered the dorsal surface."

In a subsequent paper (7) on *Diplodinium* sensu lato they affirmed that:

> "The morphology of the Ophryoscolecidae shows a few basic differences from genus to genus."

In 1927, Dogiel (6), in his monograph on this family, attempted to introduce another criterion for the recognition of the "dorsal" side—the position of the contractile vacuoles. These are usually although not invariably situated near the macronucleus and, therefore, the "dorsal" side, defined as the side where the vacuoles are situated, nearly always coincides with the "dorsal" side as defined by the position of the macronucleus. The first criterion is obviously inapplicable to genera with numerous vacuoles arranged in transverse rows, e.g. *Ophryoscolex*, *Caloscolex*, and *Polyplastron* s. str. Moreover, this criterion is confusing when strictly applied even within the limits of the genus *Entodinium*, because in species of this genus of the "laterale" group the vacuole is displaced ventrad and has shifted across the left body surface

nearly 90° from the macronucleus. Dogiel's criterion of the "dorsal" side as the side where the vacuoles are situated was probably intended to circumvent the difficulties arising from the identification of the dorsal side of *Diplodinium* s. str. (Dogiel's subgenus *Anoplodinium*), in which, as Kofoid and MacLennan (17) state, the macronucleus is located "near the middle of the right surface". Although the use of the position of the vacuoles as a criterion for the identification of the "dorsal" side of *Diplodinium* s. str. made its location in this particular genus unambiguous, it created serious difficulties in the identification of the dorsal side in the genera *Ophryoscolex* and *Caloscolex* and tended to obscure the heuristic value of the unusual position of the macronucleus in *Diplodinium* s. str. The designation of the nuclear side of *Entodinium* as the "dorsal" side, now generally accepted, is of comparatively recent origin. Von Stein (25, 26) and Schuberg (24) designated as "dorsal" the body side of *Entodinium* called at present "right side". It must be remembered that these early authors described as "ventral" the side of *Entodinium* to which the mouth is slanted; and as it is slanted (in terms of present-day nomenclature) both ventrad and to the left, at least two sides of the body could, with equal right, be termed "ventral." *Entodinium* and the vast majority of ciliates are asymmetrical organisms, swimming in spirals, the body rotating on its own axis, and simultaneously revolving around the axis of progression. It is clear that the designation "dorsal side", when applied to Ophryoscolecidae, is only a convention. This term, transferred from the morphology of bilaterally-symmetrical organisms into the morphology of the Ophryoscolecidae nearly a century ago, has carried with it not only its direct meaning but also a complex of associated ideas which, consciously or unconsciously, were accepted by all subsequent students of these ciliates. The term "dorsal side" implied the notion of permanency to the side termed "dorsal." The belief in the immutability of the "dorsal" side of the Ophryoscolecidae resulted in the usage of depicting representatives of this family always from the "same" side—the right side of *Entodinium* and the so-called "right" side of the higher Ophryoscolecidae. Dogiel (6) says:

> "Um den Vergleich der verschiedenen Formen zu erleichtern, haben wir alle Vertreter der Familie von einer und derselben, und nämlich von der rechten Seite abgebildet. Ich würde sämtlichen Ophryoscolecidenforschern raten, diesem Beispiel zu folgen. An der rechten Körperseite sind die meisten Organellen der Ophryoscolecidae gelagert (wie der Kernapparat, Schlund, Afterröhre, Skelett), während die linke Körperhälfte hauptsächlich vom Entoplasmasack eingenommen ist."

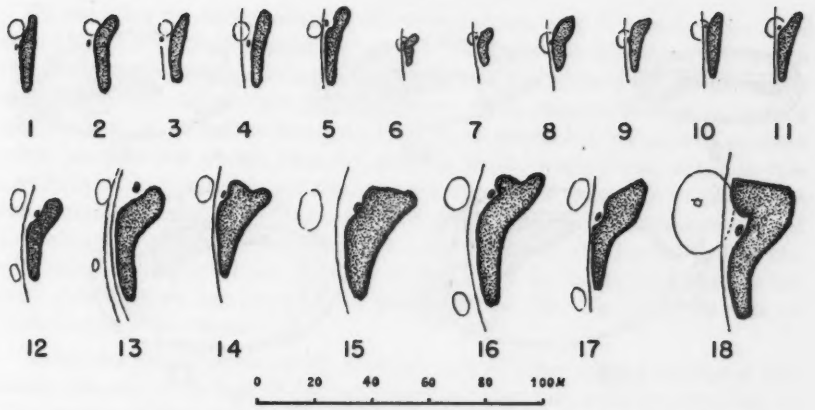
This is true in respect of the higher Ophryoscolecidae. However, the custom of depicting these ciliates from the "right" side resulted in comparing the right side of *Entodinium* with the "right" side of the higher Ophryoscolecidae and lead, therefore, to lack of interest in such important structures of *Entodinium* as the left lateral groove and the position of the contractile vacuole. This is why the obvious similarity between the left lateral groove of *Entodinium*

and the "right" dorsal furrow of *Diplodinium* s. str. has remained unnoticed, as has the close similarity of the sequence of organelles on the left body side of *Entodinium* on the one hand and the "right" body side of *Diplodinium* on the other (21).

The displacement of structures hitherto regarded as markers of body sides in the process of evolution of the Ophryoscolecidae creates considerable difficulties in the morphological terminology of this group. It is obvious that the terms "dorsal" and "ventral" sides, transferred from the morphology of higher animals with body axes constantly oriented in relation to the force of gravity, are not adequate for the designation of body sides of asymmetrical, rotating organisms. Creation of a new terminology for the designation of body sides of such organisms is a necessity. In the present paper, however, we shall use the generally accepted term "dorsal side" to designate the nuclear side of *Entodinium*. In discussing the morphology of the higher Ophryoscolecidae, we shall call their nuclear side—which from our standpoint is homologous to the ventral (antinuclear) side of *Entodinium*—the *ventral* side, adding in parentheses its usual designation ("dorsal"). This terminology stresses the arbitrariness of the terms "dorsal" and "ventral" when applied to this family. It is obviously awkward, and will be used in this paper as a palliative, pending the development of an adequate morphological terminology for asymmetrical organisms.

Displacement of Structures and the Phylogenetic Interrelations of Genera

The basic question of the comparative morphology of the Ophryoscolecidae is the interrelation between the genera *Entodinium* and *Diplodinium* s. str. As stated above (21), we came to the conclusion that *Diplodinium* originated from *Entodinium* of the "caudatum–laterale" group as the result of torsional displacement of the nuclear apparatus and the contractile vacuole from the dorsal to the left ventrolateral side. If this assumption is correct, we have to expect that the relative position and shape of structures on the dorsal side of *Entodinium* of the "caudatum–laterale" group will be similar to the configuration of these structures on the left (usually called "right") side of the body of *Diplodinium* s. str. Figs. 1 to 11 represent dorsal aspects of the nuclear apparatus and contractile vacuoles, and of the dorsal edge of the left lateral groove of several species of *Entodinium*. The lower row (Figs. 12–18) represents the nuclear apparatus and the contractile vacuoles of five species of *Diplodinium* and of one species of *Eudiplodinium* viewed from the left ("right") side. The similarity in the relative position of the represented organelles as well as in the shape of the macronucleus is obvious. It becomes still more marked in cases in which drawings are made from young specimens of *Diplodinium* and *Eudiplodinium* which possess only one contractile vacuole (Figs. 14–15, 18). The similarity between the relative position of the edge of the left lateral groove in *Entodinium* and the "right dorsal" furrow in *Diplodinium* is striking. The resemblance of the dorsal aspect of *Entodinium*



FIGS. 1–11. "Dorsal" view of the nuclear apparatus, left lateral groove, and contractile vacuole of *Entodinium*.

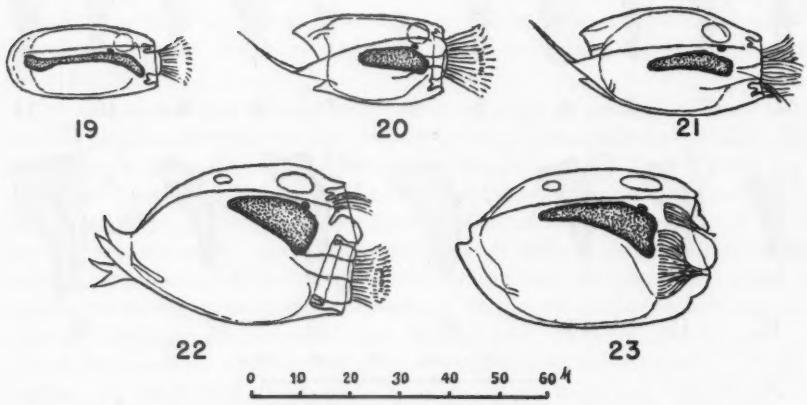
FIGS. 12–18. "Right" lateral view of the nuclear apparatus, "right" dorsal furrow, and contractile vacuoles of *Diplodinium* and *Eudiplodinium*.

FIGS. 1–5. *E. caudatum*
FIGS. 6–7. *E.* cf. *loboso-spinosum*
FIGS. 8–9. *E. rectangulatum*
FIGS. 10–11. *E. simulans* f. *caudatum*
FIG. 12. *Diplodinium dentatum*
FIG. 13. *D. anacanthum*
FIG. 14. *D. minor*
FIGS. 15–16. *D. crista-galli*
FIG. 17. *D. penthacanthum*
FIG. 18. *Eudiplodinium maggii*

All drawings were made with the aid of a camera lucida from stained and mounted material from sheep and Jamnapari goats collected in Rawalpindi, Western Pakistan, in 1950–54.

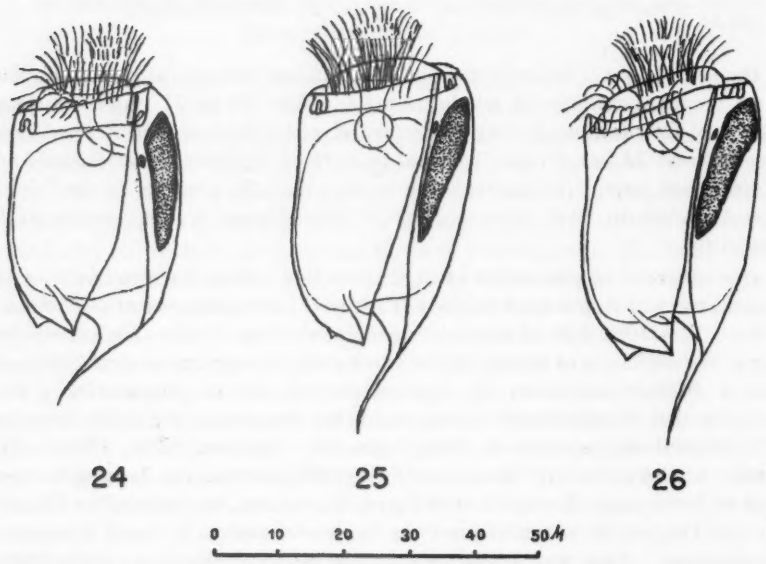
to the left ("right") lateral aspect of *Diplodinium* becomes still more obvious if we compare outlines of whole animals. Figs. 19 to 21 represent dorsal aspects of *Entodinium* (*E. longinucleatum*, *E. rectangulatum*, and *E. caudatum*). Figs. 22 and 23 left ("right") lateral aspects of *Diplodinium crista-galli* and *Diplodinium laeve*. (It is interesting to note that the presence of the "right" lateral furrow in both these species of *Diplodinium* is not mentioned by Dogiel (6).)

The torsional displacement of structures has led to the similarity of the dorsal aspect of *Entodinium* to the left ("right") lateral aspect of *Diplodinium* s. str. This 100° shift of structures around the main body axis has resulted also in the similarity of the configuration of structures on the ventral ("dorsal") side of *Diplodinium* s. str. to that on the left side of *Entodinium*. It is probable that this similarity is responsible for the persistence in the literature of a non-existing species of *Entodinium—E. dentatum* Stein, 1859. This species was depicted by Eberlein (10) in 1895 and has not been seen since. It is probable that Eberlein's drawing of this species, reproduced by Crawley (2) and Dogiel (6), is actually a drawing of the ventral ("dorsal") aspect of *D. dentatum*. This would also explain the unusual position of the micronucleus in this "species". Its micronucleus will be projected "dorsally" or "ventrally" to the macronucleus depending on the angle under which the ventral ("dorsal") side of *Diplodinium* is viewed.



FIGS. 19–21. Dorsal aspects of *Entodinium*.
FIGS. 22–23. "Right" lateral aspects of *Diplodinium*.

FIG. 19. *E. longinucleatum*
FIG. 20. *E. rectangulatum*
FIG. 21. *E. caudatum*
FIG. 22. *D. crista-galli*
FIG. 23. *D. laeve*



FIGS. 24–26. *E. simulans*. Variations in the position of the nuclear apparatus and the shape of the left lateral groove.

On studying the morphology of *E. rectangulatum* and *E. simulans* we have seen (20) that in these species the contractile vacuole is displaced ventrad to the level of the oesophagus and separated from the nuclear apparatus by the anterior portion of the left lateral groove. The examination of numerous specimens of *E. simulans* from the faunule of sheep No. 54 as well as from other faunules has shown that the position of the nuclear apparatus in this species is slightly variable and that in some specimens the posterior end of the macronucleus is shifted ventrad (Figs. 25–26). This ventrad displacement of the macronucleus is accompanied by a narrowing of the caudal end of the left lateral groove (cf. Figs. 24, 25, and 26). The similarity between the left body side of such specimens of *E. simulans* and the "right" body side of *Diplodinium* s. str. is striking.

Comparing the structure of *E. simulans* and of other related species of this genus, not only with that of *Diplodinium* s. str. but also with the structure of other genera of the higher Ophryoscolecidae, we notice that the ventrad shift, which has displaced the nuclear apparatus to the left-ventral body side in *Diplodinium* s. str., is proceeding further in other genera of this family and is shifting the nuclear apparatus still closer to the ventral ("dorsal") side of these ciliates. This ventrad shift is accompanied by progressive development of the skeleton and, to a certain degree, by progressive increase in body size.

Torsion of the body in some genera of the Ophryoscolecidae was known a long time ago. Dogiel (6, p. 221) describes the torsion of the anterior part of the body of *Opisthotrichum*. Kofoid and MacLennan (18) in describing the morphology of *Epidinium* state that:

> ". . . its main longitudinal axis is twisted, the anterior end of the body being bent ventrally and twisted to the right about the main axis. This twisting is particularly clearly shown in a dorsal view and involves the position of the skeletal plates, nuclei, contractile vacuoles, and spines as well as the general contours of the body. In other genera, such as *Diplodinium*, *Eudiplodinium*, and *Ostracodinium*, the body shows very little, if any, twisting around the main axis."

In describing the skeleton of *Caloscolex*, Dogiel (6, p. 212) stated that:

> "Der ganze Skelettkomplex wird um den Pharynx, wie um eine Längsachse, gedreht, so dass die rechte Platte von *Ophryoscolex* bei *Caloscolex* an der dorsalen Schlundseite zu liegen kommt."

Both the torsion of the body and the torsional displacement of structures are, however, not accidental morphological traits of some genera of the Ophryoscolecidae, but the basic evolutionary trends in the phylogeny of this family. The higher Ophryoscolecidae originated from ancestors similar to existing species of the "laterale" group of *Entodinium* as a result of torsional displacement of structures, which produced a new type of organization, endowed with high potential for further evolution. This major event in the phylogeny of the Ophryoscolecidae has to be regarded as an aromorphosis,

breaking way for further progressive development of the group. Although Dogiel (9, p. 429) was unaware of the role of torsion in the evolution of the Ophryoscolecidae, he wrote:

> "Examining the picture of these complications (in the evolution of the family) we notice that they comprise such a diversity of characters, and are accompanied by such an intensification of functions, that the whole ladder of the progressive changes in the course of evolution of this family in some way does not fit in the frame of idioadaptation."

The relations between different genera of the Ophryoscolecidae and their position in the dendrogram of the family can be determined on the basis of the following characters:

1. the degree of torsional displacement, that is, the degree of the ventrad shift of the nuclear apparatus and of the vacuoles;
2. the distance between the oral and the ventral ("dorsal") zone of membranelles;
3. the development of the skeleton;
4. the grade of polymerization of organelles;
5. the size of the body.

A dendrogram of the family Ophryoscolecidae, based on these criteria, is represented in Fig. 27. The most primitive genus of this family is *Entodinium* (2, 6). It has only one zone of membranelles (the adoral) and one contractile vacuole.

This genus contains over 90 species, some of which, however, may prove to be merely forms belonging to environmentally plastic species. Although the family Ophryoscolecidae (with the exception of the Polydiniinae) is basically parasitic in ruminants (Pecora and Tylopoda), the genus *Entodinium* contains two species parasitic in rodents (*E. lemmi* and *E. paludicolae* Kopperi, 1935 (19)) and one species inhabiting the colon of the Indian rhinoceros (*E. insolitum* Watson, 1945 (28)). All other species parasitize ruminants and, with the exception of one species found in camels (*E. ovumraje* Dogiel, 1926 (5)), are confined to hosts of the suborder Pecora.

All species of *Entodinium* are asymmetrical, the asymmetry being an ancestral character of this genus. Bilateral symmetry, at least in the outer shape of animals, is imposed on them by the requirement of locomotion in straight lines, and probably was never proper to the ancestors of the Ophryoscolecidae. Many species of *Entodinium* obviously possess a body shape adapted for spiraling, their spines, flanges, and grooves being spirally contorted. Such adaptations are very prominent in *Entodinium* of the *biconcavum* group, and especially in *E. tricostatum*, but are more or less expressed in all species of this genus. Effective spiraling may facilitate penetration of *Entodinium* into lumps of food swallowed by the host and thus be of survival value for these ciliates. Although spiral structure is more or less marked in all species of *Entodinium*, the ventrad displacement of the contractile vacuoles is present in only four species of this genus: *E. rec-*

tangulatum, *E. laterale* (16), *E. simulans* (20), and probably in *E. ovale*, described by Jameson (11). In a small percentage of specimens of *E. simulans* the nuclear apparatus also becomes displaced ventrad (Figs. 25–26).

According to our conception, *Diplodinium* originated from ancestral forms similar to *E. simulans* as a result of the ventrad shift of the contractile vacuole, the left lateral groove, and the nuclear apparatus. A hypothetical ancestral form of *Diplodinium* is represented in Fig. 27, No. 7. In this form, which was discussed in more detail elsewhere (21), the anterior end of the left lateral groove has distorted the outer adoral lip and thus formed the operculum. Its torsional displacement has pushed ventrad the outer row of membranelles of the adoral zone, which, however, is still continuous with the rest of this zone. Further torsion severed the displaced row of membranelles from the adoral zone. The torsional displacement of the left lateral groove and of the nuclear apparatus has thus led to partial detorsion of the adoral zone and to its division into two zones—the adoral and the so-called "dorsal". To avoid as far as possible the use of the term "dorsal" we shall call this last zone the metoral zone. Thus the genus *Diplodinium* originated—a genus differing from *Entodinium* not only in its structure but in its size and food habits. Most species of *Diplodinium* are larger than an average *Entodinium* and ingest plant fibers, which they digest with the aid of cellulolytic ferments. The increase in body size, permitting the ingestion of larger particles of cellulose, may be of survival value for larger individuals and provide a base for the progressive increase in body size in the evolution of the cellulose-ingesting genera.

In the genus *Diplodinium* s. str. (*Diplodinium* Schuberg emend. Kofoid and MacLennan) the nuclear apparatus is situated near the middle of the left ("right") body surface (Fig. 27, No. 8). As the grade of torsion decreases from the equator towards the anterior pole of the body, the macronucleus becomes flexed and has assumed the shape of a boomerang. The left lateral groove persists as a narrow furrow situated between the contractile vacuoles and the nuclear apparatus. Owing to the torsion of the endoplasmic sac the anus opens into the posterior portion of the left lateral groove. The ventrad shift of the dorsal margin of this groove has transformed its posterior portion into a wide funnel into which the anus opens. The anus of *Diplodinium* s. str. and of other genera of the higher Ophryoscolecidae is, therefore, homologous to the anus of *Entodinium* plus the caudal part of the left lateral groove. *Diplodinium* s. str. lacks a skeleton, a feature which it shares with only one genus of the higher Ophryoscolecidae—*Eodinium* Kofoid and MacLennan, 1932 (17).

This last genus (Fig. 27, No. 11) comprises a few small species in which the nuclear apparatus is shifted much farther ventrad ("dorsad") as in *Diplodinium* s. str. Accordingly this genus is more advanced than *Diplodinium* s. str. and may have originated from the latter as the result of further ventrad shift of the nuclear apparatus without the formation of a skeleton. It is also possible to regard this genus as being derived from a higher one, e.g.

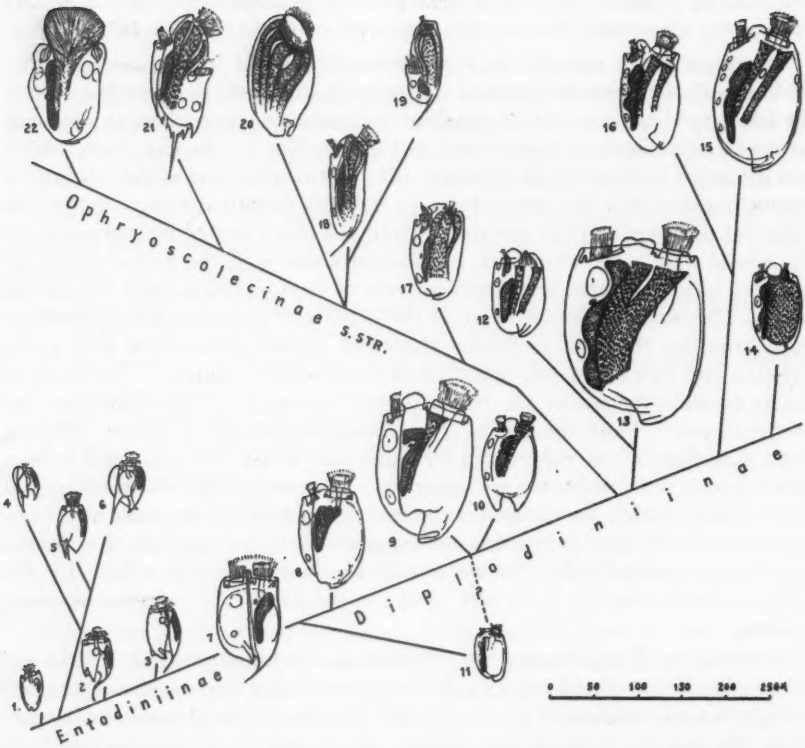


FIG. 27. All figures drawn at *ca.* ×133. No. 11 adapted from Kofoid and MacLennan (17). Nos. 19, 20, and 22—from Dogiel (6). All others are camera lucida drawings of stained and mounted material, collected by the author in Rawalpindi, Western Pakistan, from the rumina of *Bubalus bubalus* (Nos. 4–6, 14) and those of domestic sheep and goats (all other figures).

No. 1. *Entodinium nanellum* Dogiel, 1923. No. 2. *E. rectangulatum* Kofoid and MacLennan, 1930. No. 3. *E. simulans* Lubinsky, 1957. No. 4. *E. indicum* Kofoid and MacLennan, 1930. No. 5. *E. bifidum* (Dogiel, 1927). No. 6. *E. tricostatum* Kofoid and MacLennan, 1930. No. 7. Hypothetical ancestor of the higher Ophryoscolecidae. No. 8. *Diplodinium laeve* Dogiel, 1927. No. 9. *Eudiplodinium maggii* (Fiorentini, 1889). No. 10. *Eremoplastron dilobum* (Dogiel, 1927). No. 11. *Eodinium lobatum* Kofoid and MacLennan, 1932. No. 12. *Diploplastron affine* (Dogiel and Fedorowa, 1925). No. 13. *Metadinium tauricum* (Dogiel and Fedorowa, 1925). No. 14. *Ostracodinium gracile* (Dogiel, 1925). No. 15. *Polyplastron multivesiculatum* (Dogiel and Fedorowa, 1925). No. 16. *Elytroplastron bubali* (Dogiel, 1928). No. 17. *Enoploplastron triloricatum* (Dogiel, 1925). No. 18. *Epidinium caudatum* (Fiorentini, 1889). No. 19. *Opisthotrichum janus* (Dogiel, 1923). No. 20. *Epiplastron africanum* (Dogiel, 1925). No. 21. *Ophryoscolex caudatus* Eberlein, 1895. No. 22. *Caloscolex camelinus* Dogiel, 1926.

Metadinium, as a result of a secondary loss of skeleton and decrease in body size. In any case, *Eodinium* is not the most primitive genus of the higher Ophryoscolecidae as its name may suggest.

Eremoplastron Kofoid and MacLennan, 1932 (17) (Fig. 27, No. 10) is the most primitive genus of the family possessing a skeleton. This skeleton is represented by one narrow plate, the primitiva. Otherwise it closely resembles *Diplodinium* s. str.; the nuclear apparatus has not yet reached the ventral side of the body and the left lateral groove still persists as a narrow furrow situated between the nuclear apparatus and the contractile vacuoles; this furrow is usually shallower than in *Diplodinium* s. str. The macronucleus is more or less straight but slanted to the main body axis, the posterior end of the macronucleus being much closer to the ventral side ("dorsal") than the anterior. The trend to the production of caudal armature is far less expressed in *Eremoplastron* than in *Diplodinium* s. str.

The genus Eudiplodinium (Fig. 27, No. 9) is closely related to *Eremoplastron*, differing from it only in the shape of the macronucleus, which cannot be regarded as a good generic character. In this respect one may agree with Wertheim (29), who proposed to fuse *Eremoplastron* with *Eudiplodinium* s. str. into one genus—*Eudiplodinium* Dogiel emend. Kofoid and MacLennan emend. Wertheim.

Diploplastron Kofoid and MacLennan, 1932 (Fig. 27, No. 12) possesses two skeletal plates, the primitiva and carina; its macronucleus, however, is still at a considerable distance from the ventral body side and is not in contact with the vacuoles. The left lateral furrow is smoothed and almost absent.

In *Metadinium* Awerinzew and Mutafowa, 1914 emend. Kofoid and MacLennan, 1932 (Fig. 27, No. 13) the skeleton is represented by one wide plate split in two in its anterior part or by two comparatively wide plates. The macronucleus is shifted ventrad farther than in *Eremoplastron* and is partially pressed in between the vacuoles. The left lateral furrow is absent. The average size of species of *Metadinium* is much larger than that of *Diplodinium* and *Eremoplastron*.

A further stage in the shift of the nuclear apparatus is observed in *Ostracodinium* Dogiel, 1927 emend. Kofoid and MacLennan, 1932, in which the nuclear apparatus is closely pressed against the ventral body side ("dorsal side") and has displaced the contractile vacuoles to the right ventral ("left dorsal") part of the body. Only in *Ostracodinium gracile* (Fig. 27, No. 14), which is probably a primitive species, does the shape of the macronucleus and the position of the contractile vacuoles in its depressions resemble conditions typical of the genus *Metadinium*. The skeleton is represented by one very wide and long skeletal plate, extending under the entire left ("right") body surface and transgressing to the dorsal ("ventral") side. Species with three skeletal plates, differing from all other species of this genus also in some other respects, have been separated by Kofoid and MacLennan (17) into the genus *Enoploplastron* (Fig. 27, No. 17).

In the genus *Polyplastron* Dogiel, 1927 emend. Kofoid and MacLennan, 1932 the nuclear apparatus is still situated at some distance from the ventral ("dorsal") side of the body (Fig. 27, No. 15). This genus possesses a complex skeleton, consisting of five plates—two comparatively wide ones on the left ("right") side of the body and three narrow, inconspicuous ones on the right ("left") side. The contractile vacuoles are numerous and situated not only near the nuclear apparatus but also on the left ("right") side of the body. The high degree of complexity is achieved in this genus not by further torsion but by an increased number of organelles (skeletal plates and contractile vacuoles). The same applies to the closely related genus, *Elythroplastron* Kofoid and MacLennan, 1932 (Fig. 27, No. 16), which possesses only three contractile vacuoles situated near the macronucleus and three skeletal plates, two on the left ("right") side of the body and one on the right ("left") side of it.

All the genera described above possess several common characters: their adoral and metoral ("dorsal") zones of membranelles are situated approximately at the same level; the multitude of their shapes depends mainly on the degree of torsional displacement of structures and on the polymerization of organelles. In another group of the higher Ophryoscolecidae to these factors is added the elongation of the anterior part of the body between the oral and the metoral ("dorsal") membranelle zones.

One of the primitive genera of this second group is *Epidinium* Crawley, 1923 (Fig. 27, No. 18). In this genus the anterior part of the body, the "prezonal part" as Dogiel calls it, has grown; the body has become elongated and the operculum stretched and smoothened. As a result of this growth the metoral ("dorsal") zone of membranelles is situated about one-fourth of the body length behind the oral zone. *Epidinium* has a highly developed skeleton consisting of three skeletal plates. This skeleton closely resembles that of *Enoploplastron* Kofoid and MacLennan, 1932 (Fig. 27, No. 17). This last genus, consisting of only two species, has a skeleton composed of three plates, which in *Enoploplastron confluens* (Dogiel, 1925) are fused together. Its metoral membranelle zone ("dorsal zone") is situated *behind* the adoral zone and the operculum is flattened and elongated. It is probable that this genus is related to the ancestors of *Epidinium*. The description of a new form of *Enoploplastron*, provided with a long caudal spine, similar to the spine of *Epidinium—Enoploplastron triloricatum* f. *dissimilis* (Moriggi, 1950) (23)—supports this conception. The exact position of this genus, however, within the Ophryoscolecidae is not clear.

Other genera of this group (*Ophryoscolex* Stein, 1859, *Epiplastron* Kofoid and MacLennan, 1933, *Opisthotrichum* Buisson, 1923, and *Caloscolex* Dogiel, 1926) probably originated from ancestors similar to *Epidinium*. The present writer has had no opportunity of examining representatives of the last three genera. The figures of *Opisthotrichum*, *Epiplastron*, and *Caloscolex* (Fig. 27, Nos. 19, 20, 22) are taken from Dogiel (6), and brought to the scale of the other figures in Fig. 27.

According to Kofoid and MacLennan (18) *Epiplastron* (Fig. 27, No. 20): "appears to be an offshoot from *Epidinium* along the line of increase in the number of skeletal plates from three to five and an increase in size of the whole skeletal complex."

Opisthotrichum Buisson, 1923 (Fig. 27, No. 19) probably originated from Epidinium-like ancestors as the result of caudad displacement of the short metoral ("dorsal") zone, which has pushed caudad the nuclear apparatus and the contractile vacuoles. The skeleton is represented by one very wide twisted skeletal plate.

One of the most highly organized genera of the Ophryoscolecidae is the type genus of the family—*Ophryoscolex* Stein, 1859 (Fig. 27, No. 21). It probably originated from *Epidinium* by way of further development of the anterior body part, resulting in the shift of the metoral zone ("dorsal zone") closer to the equator, in the lengthening of this zone, in the increase in the number of contractile vacuoles, and in further development of the caudal spination, which reaches the highest degree of complexity in this genus.

Caloscolex Dogiel, 1926 (Fig. 27, No. 22) differs from *Ophryoscolex* in possessing a higher degree of torsion of the skeleton (6, p. 212) and a longer metoral ("dorsal") membranelle zone, and in having a lesser degree of development of the caudal spination and only one transverse row of contractile vacuoles.

Discussing the comparative morphology of the Ophryoscolecidae we came to the conclusion that in the course of the evolution of this family the nuclear apparatus and the contractile vacuole have shifted from the dorsal side of *Entodinium* to the "dorsal" side of the higher Ophryoscolecidae. This conclusion was based on the comparison of numerous species belonging to different genera of this family. In describing the changes in position of the nuclear apparatus in the process of evolution of these ciliates, we have used the generally accepted term "dorsal side" to designate the nuclear side of *Entodinium*. This body side can be termed "primary nuclear site" as opposed to the "secondary nuclear site" of the higher Ophryoscolecidae. In the course of daily work, in which comparative material is absent, it is often important to decide whether the nuclear apparatus of a given ciliate is situated in the primary or the secondary nuclear site. This is easy to do when one remembers that in all genera of the Ophryoscolecidae, primitive or advanced, the micronucleus is situated ventrally to the macronucleus. If, in the lateral aspect of an ophryoscolecid ciliate, the macronucleus is situated between the micronucleus and the nearest body outline, the nuclear apparatus is at the primary nuclear site; if the micronucleus is situated between the macronucleus and the nearest body outline, the nuclear apparatus is at the secondary nuclear site.

Grouping of Genera Within the Family Ophryoscolecidae

The genera discussed above, despite their high diversity, are included in one family, the Ophryoscolecidae, and are not grouped into subfamilies,

although they obviously constitute several natural groups. Within the Ophryoscolecidae Kofoid (15) has established one subfamily, the Polydiniinae, to include two genera of peculiar ciliates (*Polydinium* and *Elephantophilus*) from an Indian elephant. The remaining genera were never subdivided into or united in a subfamily, although Bhatia (1, p. 275) stated that the subfamily Ophryoscolecinae was established by Kofoid in 1935 and characterized this subfamily as follows:

> "Subfamily Ophryoscolecinae Kofoid, 1935. Ophryoscolecidae with an adoral membranelle zone and with or without a dorsal zone. One to five skeletal plates present. Contractile vacuoles usually one to twelve in number, located on the dorsal side adjacent to the macronucleus."

This characteristic of the subfamily Ophryoscolecinae obviously excludes from it all genera without skeleton, that is, *Entodinium*, *Diplodinium* s. str., and *Eodinium*. On the other hand, the statement that the contractile vacuoles are located on the dorsal side adjacent to the macronucleus would exclude from this subfamily the genera *Ophryoscolex*, *Caloscolex*, and *Polyplastron*. A comparison of this characteristic of the subfamily Ophryoscolecinae, ascribed by Bhatia (1) to Kofoid (15), with the text of Kofoid's paper shows that he merely discussed in it the morphological characters of the *family* Ophryoscolecidae. He states:

> "The Ophryoscolecidae form a compact but structurally diversified assemblage characterized by an adoral membranelle zone, a heavy peripheral pellicle, permanently located excretory vacuoles, and a system of from *one to five vacuolated "skeletal" plates* arranged peripherally to the pharynx and projecting posteriorly along the side of the macronucleus. These plates are characteristic of all but a few of the most primitive genera of this family. A few of the higher genera, such as *Epidinium*, *Diplodinium*, and *Ophryoscolex*, of the Ophryoscolecidae, add to the adoral zone of membranelles a second, always dorsal, membranelle zone, having no relationship to the feeding function, but accessory to the locomotor function."

And further:

> "The excretory *vacuoles, from one to twelve in number* are located in the dorsal side adjacent to the macronucleus except in those cases where the vacuoles are numerous, in which instances they have a wider distribution."

It is obvious that Bhatia compiled the diagnosis of the subfamily Ophryoscolecinae, the establishment of which he ascribes to Kofoid, from sentences taken from Kofoid's description, not diagnosis, of the *family* Ophryoscolecidae. Kofoid cannot, therefore, be regarded as author of the diagnosis of the subfamily Ophryoscolecinae, nor can Bhatia, as he obviously compounded it from Kofoid's discussion of morphological traits of the *family* Ophryoscolecidae. Moreover, Bhatia badly distorted Kofoid's discussion by omitting several important points concerned with genera devoid of skeleton and with the

position of the contractile vacuoles in some higher genera of the family. The whole incident with the diagnosis of the subfamily Ophryoscolecinae can, therefore, be dismissed as a vexatious misunderstanding.

With the exception of the Polydiniinae all genera of the Ophryoscolecidae discussed in this paper can be grouped into three subfamilies, namely:

(1) Subfamily ***Entodiniinae*** subf. nova

One membranelle zone, one contractile vacuole. In side view the macronucleus between the micronucleus and the nearest body outline.

Type genus: *Entodinium* Stein, 1859.

(2) Subfamily ***Diplodiniinae*** subf. nova

Two membranelle zones situated approximately in one transverse plane. Two or more contractile vacuoles. In side view the micronucleus situated between the macronucleus and the nearest body outline. Skeleton absent or present. Body more or less flattened.

Type genus: *Diplodinium* Schuberg, 1888 emend. Kofoid and MacLennan, 1932.

Other genera:

Eodinium Kofoid and MacLennan, 1932,
Eremoplastron Kofoid and MacLennan, 1932,
Eudiplodinium Dogiel, 1927 emend. Kofoid and MacLennan, 1932,
Diploplastron Kofoid and MacLennan, 1932,
Metadinium Awerinzew and Mutafowa, 1914 emend. Kofoid and MacLennan, 1932,
Polyplastron Dogiel, 1927 emend. Kofoid and MacLennan, 1932,
Elytroplastron Kofoid and MacLennan, 1932,
Ostracodinium Dogiel, 1927 emend. Kofoid and MacLennan, 1932,
Enoploplastron Kofoid and MacLennan, 1932.

(3) Subfamily ***Ophryoscolecinae*** subf. nova

Two membranelle zones situated in different transverse planes. Two or more contractile vacuoles. In side view the micronucleus situated between the macronucleus and the nearest body outline. Skeleton present. Body more or less cylindrical.

Type genus: *Ophryoscolex* Stein, 1859.

Other genera:

Epidinium Crawley, 1923,
Opisthotrichum Buisson, 1923,
Caloscolex Dogiel, 1926,
Epiplastron Kofoid and MacLennan, 1933.

These three new subfamilies differ considerably in the grade and character of torsion as well as in other respects. In the Entodiniinae, the most primitive subfamily, the torsion is absent or only slightly marked (in the "caudatum–laterale" group of *Entodinium*). The nuclear apparatus is situated at the primary nuclear site.

In the Diplodiniinae the nuclear apparatus and the contractile vacuoles are shifted clockwise across the left ("right") body side. The shift of the

macronucleus in different genera of this subfamily ranges from about 100° (in *Diplodinium* s. str.) to 180° (in *Ostracodinium*), the development of the skeleton being directly proportional to the grade of torsional displacement of the nuclear apparatus. The metoral membranelle zone remains approximately in the same transverse plane as the adoral zone. Only in *Enoploplastron* is the metoral zone shifted slightly caudad, a trend which becomes prominent in the evolution of the subfamily Ophryoscolecinae.

The third subfamily, the Ophryoscolecinae, is characterized by a high degree of torsion, considerable development of the skeleton and of the caudal spination, and chiefly by the elongation of the interzonal (or in Dogiel's terms, "prezonal") part of the body. Whereas in the Diplodiniinae the highest degree of torsion takes place near the middle of the body (the equator), in the Ophryoscolecinae it is the anterior, interzonal part of the body which undergoes maximum twisting. The nuclear apparatus, which is situated in this family caudad to the zone of maximal twisting, is, at least in the primitive genera of this subfamily, displaced clockwise less than 180°.

It can be surmised that the Ophryoscolecinae branched off from the Diplodiniinae after the development of a fairly complex skeleton, but before the macronucleus had been pressed between the vacuoles, that is, before the advent of the genus *Metadinium*. Contradicting this suggestion, however, is the fact that *Epidinium*, one of the primitive genera of the Ophryoscolecinae, has a skeleton composed of three plates closely resembling that of *Enoploplastron triloricatum* (Dogiel, 1925), which Dogiel placed in the genus *Ostracodinium*. It is possible, therefore, that the Ophryoscolecinae branched off from the Diplodiniinae later than is shown in Fig. 27.

The subfamily Polydiniinae Kofoid, 1935 is closely related to and probably originated from the Ophryoscolecinae. It is characterized by a very high degree of torsion, far exceeding 180°, which is the maximal torsion observed in the Diplodiniinae. Both genera of the Polydiniinae inhabit the colon of the Indian elephant, thus differing from members of the previous three subfamilies which are basically parasites of ruminants.

In Fig. 27 (drawn at a magnification of *ca.* ×133), the genera are represented by species approximating the average size of species of any given genus. The plate shows clearly the increase in the average body size of the Ophryoscolecidae in the course of their evolution. However, the largest sizes are attained not by representatives of the genera with the most complex organization but by species of *Eremoplastron* and *Metadinium*, both comparatively primitive genera of Diplodiniinae (6, p. 26). Dogiel surmised that the increase in size of the Ophryoscolecidae in the course of their evolution depended on their parasitic way of life. This does not explain, however, the difference in body sizes in different subfamilies of the Ophryoscolecidae. Later, Dogiel (9) pointed out that the rumen can be compared in its volume with that of a small natural water basin and that the Ophryoscolecidae thus live under conditions similar to those of free-living organisms, except that in the host's environment food is more abundant. It might be surmised that the increase in size of the

Ophryoscolecidae in the course of their evolution represents a particular case of the law of increase of size in phyletic branches of Cope–Depéret. This law was established by Cope, however, on the basis of studies of evolution of vertebrates and its inapplicability to the evolution of at least several branches of protozoa was pointed out by Depéret (3). It is probable that the differences in the increase in body size in the evolution of different branches of the Ophryoscolecidae depend on differences in food habits. Whereas the increase in size may be of survival value for cellulose-feeders and predators, it may be of no advantage to bacteria- or starch-feeders. This would explain the large body sizes attained by some genera of Diplodiniinae, which are predominantly cellulose-feeders, and the moderate sizes of the Ophryoscolecinae, which are basically starch-feeders.

Another remarkable character of the evolution of the Ophryoscolecidae is the dependence of the development of the caudal spination on the degree of torsional displacement of the nuclear apparatus. Only primitive genera of the Diplodiniinae with nuclear apparatus situated at a considerable distance from the secondary nuclear site ("dorsal side"), for example *Diplodinium* s. str., and to a less degree *Eremoplastron*, have species with well-developed caudal spination. Genera with nuclei near the secondary nuclear site, for example *Metadinium*, *Diploplastron*, *Polyplastron*, and *Elytroplastron*, have no armed species or have only a few species with poorly-developed spination, as for example *Ostracodinium*. A similar regularity exists in the subfamily Ophryoscolecinae; genera with the nuclear apparatus near the middle of the left ("right") side of the body, *Epidinium* and *Ophryoscolex*, have well-developed caudal spination, whereas genera which have undergone further torsion, for example *Caloscolex* and *Opisthotrichum*, as well as the Polydiniinae, have poorly-developed caudal spination.

Torsional displacement of structures is the leading trend in the phylogeny of the Ophryoscolecidae, a trend determining the basic morphological features of this family. As a result of it the nuclear apparatus and the contractile vacuole were shifted from the dorsal side of primitve Ophryoscolecidae to the ventral side of the higher genera. The higher Ophryoscolecidae, Diplodiniinae and Ophryoscolecinae, can be united in the group Ventronucleata and opposed to the primitive group Dorsonucleata, comprising only one subfamily, the Entodiniinae. Leaving aside the Polydiniinae, possessing a high degree of torsion, we can affirm that in all Ophryoscolecidae, higher as well as lower, the macronucleus is situated dorsally to the micronucleus, a character which helps to distinguish Dorsonucleata from Ventronucleata at first glance. These relations change and the structures become much more complicated when the torsion exceeds 180°, as is the case in Polydiniinae. From this standpoint it would be interesting to study the morphology of other ciliates and, in the first place, of other groups of Entodiniomorpha. A detailed study of the genera *Spirodinium*, *Cochliotoxum*, and *Tetratoxum* of the Cycloposthiidae would probably throw new light on the origin of this most interesting family and on its relations with the Ophryoscolecidae.

Some Conjectures and Problems

In comparing the morphological characters of *Entodinium* with those of the higher Ophryoscolecidae we have traced the torsional displacement of structures in the course of the phylogeny of these ciliates. We do not know the causes of this process, as we do not know the causes of so many phylogenetic phenomena, but it is hard to resist the temptation to put forward some hypothetical explanations. In trying to analyze the dynamics of the displacement of structures in the phylogeny of the Ophryoscolecidae it is natural to regard it, to use d'Arcy Thompson's (27) words:

> "as related to or resulting from the physical causes inherent in the entire system of forces to which the whole has been exposed, and under whose influence it has come into being."

Already a superficial study of the mode of life of these ciliates reveals the remarkable fact that in swimming they rotate clockwise whereas the structures are displaced counterclockwise. Their displacement can thus be described as the lagging of these structures behind the rotation of the body. This displacement was extremely slow—the transformation of ancestral *Entodinium* into *Diplodinium* may have necessitated a time span of the order of a geologic period.

The occurrence of repeated torsions and detorsions in the life cycle of apostomatous ciliates, resulting in coiling and uncoiling of the ciliature, was studied by Chatton and Lwoff and by Lwoff (22). A temporary displacement of structures in individual trichomonads and devescovinid flagellates was described by Kirby (14). In *Devescovina lemniscata* Kirby (12) observed: "the rotation of entire systems of organelles in a counterclockwise direction as viewed from the rear". In *Macrotrichonympha restis* he (13) observed: "The axostyle, parabasal body, and nucleus moved as a unit, the relative position of these parts undergoing no alteration". These phenomena, occurring in flagellates with semifluid nude cytoplasm, are of short duration and but slightly reminiscent of the phylogenetic phenomena in the Ophryoscolecidae, ciliates invested with a fairly resistant pellicle and having a constant body shape. We may conjecture, however, that the displacement of structures in the phylogeny of the Ophryoscolecidae depends in some way on their manner of locomotion. The probable influence of such factors as the friction and viscosity of the medium, the specific gravity of the nuclear apparatus, the centrifugal force developed in the course of locomotion, and the precise character of this locomotion and that of the body shape will probably have to be taken into account. The role of persistence of characters produced by *persisting* environment factors in these ciliates will also have to be considered. In trying to analyze the dependence of displacement of structures in the phylogeny of the Ophryoscolecidae on their mode of life, we are forced to resort almost to guess work and at this point it is safer to stop and to say "ignoramus," but not "ignorabimus".

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NOTES

METRETOPUS BOREALIS (EATON) IN CANADA (EPHEMEROPTERA: AMETROPODIDAE)

GEORGE F. EDMUNDS, JR.[1]

The only species of the holarctic mayfly genus *Metretopus* in North America is *M. borealis* (Eaton). In the literature on North American mayflies, it has been recorded by the name *M. norvegicus* Eaton, but Brekke (2) has shown the name *norvegicus* Eaton, 1901 to be a synonym of *M. borealis* (Eaton) 1871, a name formerly assigned to *Rhithrogena*.

When McDunnough (6) reported specimens of *Metretopus* from Slave Lake, Alberta, he remarked that "The male specimen before me agrees so well with Eaton's figures and description of norvegicus that for the present I am listing the species under this name." Through the kindness of Dr. Richard Frey and Mr. L. Tiensuu of the Museum Zoologicus Universitatis, Helsinki, I have examined specimens of *M. borealis* from Finland and have compared them with the specimens in the Canadian National Collection. This comparison proves that the European and Canadian populations are conspecific and apparently indistinguishable. In addition to the two specimens (one male, one female) reported from Slave Lake (Aug. 17, 1924, O. Bryant) by McDunnough, the following additional specimens are in the Canadian National Collection: Rolla, B.C., Aug. 14, 1927, P. N. Vroom, one female; Churchill, Manitoba, July 31, 1937, W. J. Brown, three males; and Newcastle, N.B., July 6, 1928, W. J. Brown, three males. Thus this species is seen to have a widespread distribution in Canada.

There has been some disagreement as to the relationship of the mayfly genera *Metretopus* and *Siphloplecton* in mayfly classification. Lestage (5) placed the two genera in separate families (Metretopodidae and Siphloplectonidae), while Edmunds and Traver (4) have placed both genera in the subfamily Metretopodinae of the family Ametropodidae. After examination of nymphs and adults of both genera, I feel that there are two closely related, but distinct, genera involved.

The nymphs of the two genera are usually distinguished in keys by means of their maxillary palpi, stated to be two-segmented in *Siphloplecton* and three-segmented in *Metretopus* (see Needham, Traver, and Hsu (7), and Burks (3)). Actually the maxillary palpi of *Metretopus* are also two-segmented rather than three-segmented as described by Bengtsson (1).

The most reliable difference in the nymphs of the two genera is found in the shape of the terminal segment of the labial palpi. This segment is rounded in *Siphloplecton* (as seen in *S. basale* Walker), while it is expanded and truncate apically in *Metretopus* (as seen in *Metretopus borealis* from Finland).

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Contents

Page

A Technique for Estimating the Fecundity of Natural Populations of the Spruce Budworm—*C. A. Miller* - - - - - - - - - - - 1

Observations on the Life Cycles of *Diplotriaenoides translucidus* Anderson and Members of the Genus *Diplotriaena*—*Roy C. Anderson* - - - 15

Taxonomic Studies on the Genera *Aproctella* Cram, 1931 and *Carinema* Pereira and Vaz, 1933 with a Proposal for a New Genus *Pseudaproctella* n. gen.—*Roy C. Anderson* - - - - - - - - - - - 25

The Embryology of the Black Widow Spider, *Latrodectus mactans* (Fabr.)—*J. G. Rempel* - - - - - - - - - - - - - - 35

The Morphology of the Nematode *Radopholus gracilis* (de Man, 1880) Hirschmann, 1955, Parasitic in Roots of Wild Rice, *Zizania aquatica* L.—*K. C. Sanwal* - - - - - - - - - - - - - - 75

The Biology and Pathology of the Tapeworm *Taenia hydatigena* in Domestic and Wild Hosts—*G. K. Sweatman and P. J. G. Plummer* - - - - - 93

Studies on the Evolution of the Ophryoscolecidae (Ciliata: Oligotricha). I. A New Species of *Entodinium* with "caudatum," "loboso-spinosum," and "dubardi" Forms, and Some Evolutionary Trends in the Genus *Entodinium*—*G. Lubinsky* - - - - - - - - - - - - 111

Studies on the Evolution of the Ophryoscolecidae (Ciliata: Oligotricha). II. On the Origin of the Higher Ophryoscolecidae—*G. Lubinsky* - - - 135

Studies on the Evolution of the Ophryoscolecidae (Ciliata: Oligotricha). III. Phylogeny of the Ophryoscolecidae Based on Their Comparative Morphology—*G. Lubinsky* - - - - - - - - - - - - 141

Notes:

Metretopus borealis (Eaton) in Canada (Ephemeroptera: Ametropodidae) —*George F. Edmunds, Jr.* - - - - - - - - - - - 161

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